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The Effects of Vermicompost and Steel Slag Amendments on the Physicochemical Properties and Bacterial Community Structure of Acidic Soil Containing Copper Sulfide Mines

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Abstract: Acidification and heavy metal stress pose challenging threats to the terrestrial environment. This investigation endeavors to scrutinize the combined effects of vermicompost and steel slag, either singularly or in concert with Ryegrass (*Lolium perenne* L.), on the remediation of acidic soil resulting from sulfide copper mining. The findings illuminate substantial ameliorations in soil attributes. The application of these amendments precipitates an elevation in soil pH of 1.39–3.08, an augmentation in organic matter of 4.05–8.65, a concomitant reduction in total Cu content of 43.2–44.7%, and a marked mitigation in Cu bioavailability of 64.2–80.3%. The pronounced reduction in soil Cu bioavailability within the steel slag treatment group (L2) is noteworthy. Characterization analyses of vermicompost and steel slag further elucidate their propensity for sequestering Cu^{2+} ions in the soil matrix. Concerning botanical analysis, the vermicompost treatment group (L1) significantly enhances soil fertility, culminating in the accumulation of 208.35 mg kg^{-1} of Cu in *L. perenne* stems and 1412.05 mg kg^{-1} in the roots. Additionally, the introduction of vermicompost and steel slag enriches soil OTU (Operational Taxonomic Units) quantity, thereby augmenting soil bacterial community diversity. Particularly noteworthy is the substantial augmentation observed in OTU quantities for the vermicompost treatment group (L1) and the combined vermicompost with steel slag treatment group (L3), exhibiting increments of 126.04% and 119.53% in comparison to the control (CK). In summation, the application of vermicompost and steel slag efficaciously diminishes the bioavailability of Cu in the soil, augments Cu accumulation in *L. perenne*, induces shifts in the soil microbial community structure, and amplifies soil bacterial diversity. Crucially, the concomitant application of vermicompost and steel slag emerges as a holistic and promising strategy for the remediation of sulfide copper mining acidic soil.

Keywords: vermicompost; steel slag; copper; soil remediation; bacterial diversity

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1. Introduction

Mining activities have precipitated extensive ecological degradation and environmental contamination, emerging as a paramount global concern. Due to the sulfur content inherent in ore constituents, sulfide copper ores undergo insufficient separation during ore beneficiation processes. Unseparated sulfur and tailings are collectively deposited in waste piles, culminating in long-term soil exposure and pervasive issues of soil acidification and heavy metal contamination [1]. Furthermore, excessive copper (Cu) levels in the soil incite plant toxicity symptoms, severely compromising plant establishment and growth [2]. Hence, the judicious selection of efficient and cost-effective amendments for the remediation of acidic soils associated with sulfide copper mining assumes pivotal significance. Establishing a secure and stable vegetation ecosystem is of paramount importance for the ecological restoration of soils in metal-contaminated mining areas.

In recent years, a plethora of inorganic and organic amendments has found widespread application in the remediation of soils tainted with heavy metals, constituting what is known as in situ immobilization remediation. By judiciously adding amendments to the soil, this technique modulates the form of heavy metals, reducing their mobility and bioavailability. This presents an effective, cost-efficient, and sustainable remediation paradigm [3]. However, inherent disparities in the composition of various amendments may impart corresponding limitations to their efficacy in soil remediation. Notably, biochar, endowed with a meticulously defined porous structure and an abundance of oxygen-containing functional groups, exhibits commendable adsorption capabilities for soil pollutants. Nevertheless, uncertainties enveloping the aging of biochar may portend potential environmental risks, and its exorbitant cost may render it impracticable for large-scale mining restoration initiatives [4]. While the application of lime expeditiously elevates the pH of acidic soils, its aptitude to sustain soil pH equilibrium is circumscribed, rendering it susceptible to secondary acidification and potential soil compaction with protracted use [5]. Ergo, the quest for environmentally friendly and cost-effective amendments stands as a linchpin in the realization of in situ immobilization remediation of soils. Extant research posits that vermicompost, replete with organic matter and diverse amino acids, proffers superlative water retention, breathability, high fertility, and enduring fertility. They efficaciously ameliorate the physical, chemical, and biological properties of the soil. Additionally, vermicompost, with its discernibly porous structure, can serve as adept adsorbents and passivators for heavy metals [6]. As a byproduct of the steel production process, steel slag has a utilization rate of only approximately 22%. Steel slag not only burnishes soil structure and regulates soil pH but also enhances the soil's proclivity for metal ions, fomenting the formation of hydroxides. Ergo, employing steel slag as a soil amendment for the remediation of acidic soils associated with sulfide copper mining is a sagacious and sustainable choice [7]. *L. perenne,* with its potential for phytostabilization of acidic and heavy metal-contaminated soils, emerges as an eminently suitable candidate [8]. The concomitant cultivation of *L. perenne* affords a holistic evaluation of the remediation effects of vermicompost and steel slag on acidic soils associated with sulfide copper mining.

The ultimate aim of soil remediation transcends the mere reduction in the bioavailability and mobility of deleterious metals within the soil; it is paramount to elevate the health indices of the soil. The intricacies of the soil microbial community structure wield profound importance in augmenting soil functionality, representing a pivotal metric for assessing soil vitality. Consequently, a heightened focus on the nuanced alterations within soil microbial diversity becomes imperative [9]. A compendium of scholarly investigations attests that the introduction of ameliorative agents serves to augment the diversity inherent in the soil microbial framework, thereby amplifying the functional repertoire of these microorganisms. Notably, the application of steel slag manifests a conspicuous augmentation within a specific cohort of soil bacterial communities, bestowing considerable utility upon soil–plant remediation endeavors [10]. Correspondingly, vermicompost furnishes a plethora of nutrients conducive to microbial proliferation, thereby potentiating heightened microbial activity and functionality. This augmentation, in turn, engenders an enhancement in microbial respiration, culminating in a more efficacious amelioration of soil structure [11]. It is noteworthy that the extant body of literature is somewhat bereft in delving into the collective impact of vermicompost and steel slag on the structural dynamics of soil microbial communities in the co-presence of vegetation.

This investigation employs vermicompost and steel slag as soil amendments, utilizing them individually or in conjunction with *L. perenne* for the amelioration of acidic soils associated with sulfide copper mining. The outlined objectives of this research endeavor are (1) to elucidate the mechanistic intricacies of vermicompost and steel slag via comprehensive characterization analysis; (2) to scrutinize the ramifications of the combined application of vermicompost, steel slag, and *L. perenne* on the physicochemical attributes of the soil, concomitant with an examination of the bioavailability of copper (Cu); (3) to analytically ascertain the biomass and heavy metal accumulation of *L. perenne* under the influence of vermicompost and steel slag; and (4) to assess the intricate interplay between soil environmental factors and the nuanced diversity characterizing the soil bacterial community structure.

2. Materials and Methods

2.1. Tested Soil and Amendments

The soil was procured from Chengmenshan Copper Mine (115°48′32″ E, 29°41′26″ N), situated in the Chaisang District of Jiujiang City, Jiangxi Province, China. Employing a meticulous five-point sampling method, a homogeneous soil sample was extracted from the surface layer (5–30 cm). Employing the quartile method, the soil samples underwent a partition into two segments. One segment was transported to the laboratory, where it underwent air-drying, grinding, and sieving through a 2 mm nylon sieve for subsequent utilization in pot experiments. The second segment found its place of preservation in a −80 °C freezer to facilitate DNA extraction. The steel slag utilized in this experimental inquiry emanated from a local iron and steel establishment, while vermicompost was procured from Huizhou Nianhe Agricultural Technology Co., Ltd., Huizhou, China. The examination of the surface microphotographs of vermicompost and steel slag was conducted via Scanning Electron Microscopy (SEM, Zeiss EVO18, Tokyo, Japan). X-ray Diffraction (XRD, Malvern Panalytical Empyrean, San Jose, CA, USA) was instrumental in discerning the mineral composition of the steel slag. Fourier Transform Infrared Spectroscopy (FTIR, Thermo Scientific Nicolet iS20, Waltham, MA, USA) served as the analytical tool for probing the surface functional groups of vermicompost. The fundamental physicochemical parameters of the soil and amendments are presented in the following table (Table 1).

Table 1. Main physicochemical properties of the tested soil, vermicompost, and steel slag.

Properties	Tested Soil	V	S
pH	3.78	6.4	12.52
cation exchange capacity (CEC, cmol·kg^{-1})	17	na	na
soil organic matter (SOM, g·kg^{-1})	5.93	470.6	na
Total N (TN, g·kg^{-1})	0.98	16.94	na
Total P (TP, g·kg^{-1})	0.45	16.73	na
Total K (TK, g·kg^{-1})	5.61	12.88	na
Total Cu (g·kg^{-1})	2.41	na	0.02

Note: V, vermicompost; S, steel slag; na, valid values are not available.

2.2. Experimental Design

In March 2023, a potted experiment was conducted within the greenhouses of Jiangxi University of Science and Technology. Building upon the initial screening outcomes of our research group, a combination of vermicompost (4% *w*/*w*) and steel slag (2% *w*/*w*) was meticulously chosen as the amendment ratio for this investigation. White plastic pots featuring perforations at the base were filled with 2.5 kg of soil intermixed with the specified amendments. Preceding the commencement of the experiment, a thorough amalgamation of amendments and soil took place, followed by watering to achieve 70% of the maximum field water-holding capacity. A two-week equilibration period was observed. The experimental treatments comprised (1) the control soil devoid of any amendments (CK); (2) vermicompost (4% *w*/*w*) (L1); (3) steel slag (2% *w*/*w*) (L2); and (4) a combination of vermicompost (4% *w*/*w*) and steel slag (2% *w*/*w*) (L3). Each treatment was replicated three times.

L. perenne, selected for its remarkable capacity to accumulate heavy metals and manifest tolerance mechanisms to copper toxicity, served as the focal plant species in this study. Seeds of *L. perenne* were procured from the Yunguang flagship store. Fifty seeds were evenly distributed in each pot, and following germination, the seedlings were selectively thinned to maintain 30 plants per pot (all *L. perenne* within the CK treatment wilted and perished within two weeks under the duress of heavy metal stress). Daily irrigation was administered using deionized water, with soil moisture meticulously maintained at 70% of the maximum field water-holding capacity.

2.3. Plant Sampling and Analysis

After a six-month growth period, the *L. perenne* was harvested. The *L. perenne* was thoroughly washed with deionized water to remove any contaminants. The stems, leaves, and roots of the *L. perenne* were then separated and dried in an oven at 70 °C until a constant weight was achieved. The dry weight was recorded using a milligram balance. A 0.2 g dried plant sample was weighed in a conical flask and added with 5 mL of HNO_3 and 1 mL of $HClO_4$. The flask was placed on a graphite digestion device at 200 °C until the solution in the flask became colorless and transparent (this process took about 2–3 h, during which hydrogen peroxide can be added dropwise). The solution was then transferred into a 50 mL volumetric flask and diluted to the mark with distilled water. This is the extraction solution. The concentration of Cu in the extraction solution was directly determined via Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) [12].

2.4. Soil Chemical Analysis

The soil samples were from the pots, and the plant residues were eliminated. The samples were passed through a 20 mm nylon sieve, and the corresponding chemical analyses were conducted [12]. The pH of the soil suspension was measured using a glass electrode with a water-to-soil ratio of 2.5:1. The soil electrical conductivity (EC) was determined using the conductivity method with a water-to-soil ratio of 5:1. The soil organic matter (SOM) was measured using the potassium dichromate dilution heat method [13]. The cation exchange capacity (CEC) was measured using the ammonium acetate method [14]. The soil redox potential (Eh) was measured using a platinum redox electrode (In Lab Redox). The total Cu content in the soil was determined via ICP-OES, and the available Cu content in the soil was determined via Diethylene Triamine Pentaacetic Acid (DTPA) extraction inductively coupled plasma emission spectroscopy [15].

2.5. Soil Bacterial Community Diversity Analysis

Genomic DNA from the microbial community was meticulously extracted utilizing the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA), adhering to the manufacturer's prescribed instructions. The resultant DNA extract underwent scrutiny on a 1% agarose gel, and its concentration and purity were judiciously assessed using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, NC, USA). The amplification of the bacterial 16S rRNA gene's hypervariable region V3-V4 was executed with the primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) employing an ABI GeneAmp® 9700 PCR thermocycler (ABI, Los Angeles, CA, USA). Subsequently, the purified amplicons were amalgamated in equimolar proportions and subjected to paired-end sequencing on an Illumina MiSeq PE300 platform or NovaSeq PE250 platform (Illumina, San Diego, CA, USA) in strict adherence to standardized protocols, as implemented by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) [16].

2.6. Statistical Analysis

All statistical analyses were conducted using SPSS 26.0 software. Single-factor analysis of variance (ANOVA) and Duncan's multiple range test were employed for multiple comparisons (n = 3, $p < 0.05$). Graphs were created using Origin 2022 for data visualization. Additionally, symbiotic network graphs were generated using Gephi 0.9.2, and correlation heatmaps were constructed using R Studio 4.0.4.

3. Results and Discussion

3.1. Characterization of Amendments

3.1.1. SEM and FTIR Analysis of Vermicompost

SEM images vividly depict the intricately dispersed reticular architecture adorning the surface of vermicompost, presenting an exalted degree of porosity that affords a plethora of adsorption loci for the sequestration of heavy metals (Figure 1A). FTIR is an indispensable methodology for scrutinizing and evaluating of the molecular functional groups inherent in substances [17]. Via a discerning examination of the FTIR spectra of vermicompost pre- and postCu^{2+} adsorption (Figure 1B), a discernible congruity in the peak positions of the infrared spectra manifests itself, with the principal absorption bands aligning within the spectral range of 3300 to 3600 cm^{-1}. Notwithstanding, nuanced differentials in peak intensity are observed. The resonances at 3431 and 3425 cm^{-1} find attribution to the stretching vibrations of O-H in phenolic and alcoholic entities [18]. The zenith at 2928 cm^{-1} is ascribed to the stretching vibration of C-H within aliphatic moieties. The features at 1647 and 1651 cm^{-1} correlate with the stretching vibrations of C=C within aromatic compounds, serving as proxies for the aromatic compound content within vermicompost [19]. The apices at 1386 and 1412 cm^{-1} correspond with the deformation vibrations of C-H within $-CH_3$ and $-CH_2$ of aliphatic compounds [20]. Furthermore, the asymmetrical stretching vibrations of C-O within polysaccharides correlate with the peaks at 1041 and 1038 cm^{-1} [18]. In comparison to untreated vermicompost, a discernible attenuation in the intensity of the absorption band corresponding to the aromatic C=C bond is observed in vermicompost post-Cu^{2+} adsorption, imputed to the interaction between the delocalized π electrons inherent in aromatic structures and Cu^{2+} [21]. The absorption bands associated with O-H and C-O bonds also exhibit a diminution in intensity post-Cu^{2+} adsorption, with the primary impetus for this transformation potentially residing in the occurrence of chelation between Cu^{2+} and oxygen-bearing functional groups [22].

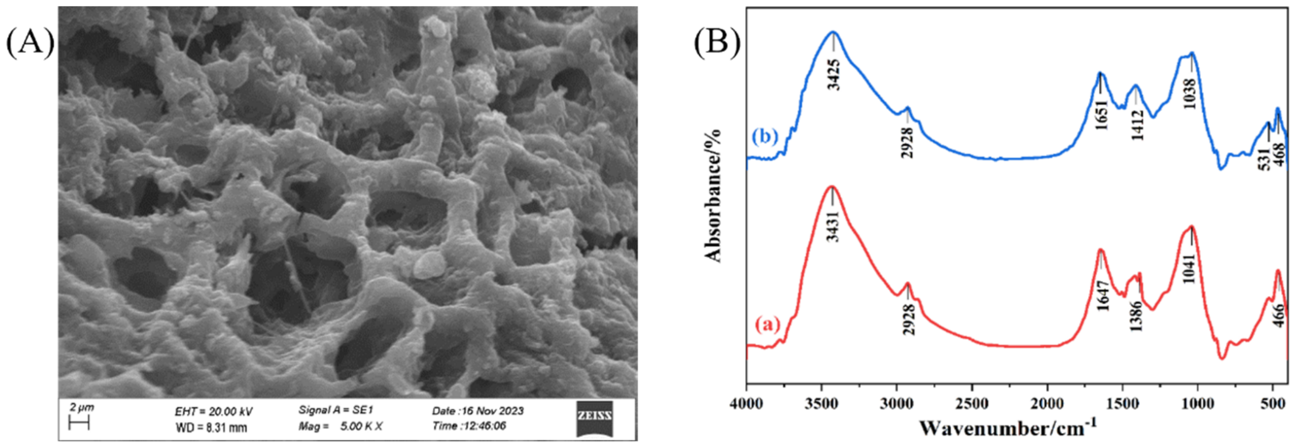

Figure 1. SEM images of vermicompost (**A**); FTIR spectra of vermicompost (a) and vermicompost after Cu^{2+} adsorption (b) (**B**).

3.1.2. SEM and XRD Analysis of Steel Slag

The SEM analysis illustrates (Figure 2A) the presence of numerous agglomerates on the surface of the steel slag, interspersed with a modicum of non-agglomerated entities, exhibiting an irregular disposition. A plethora of investigations affirms the stellar mesoporous adsorbent qualities of steel slag, characterized by robust affinities and efficacious removal rates for metallic ions [23]. The compositional scrutiny of steel slag, validated via XRD (Figure 2B), delineates predominant constituents in the form of oxygenated compounds of Fe, Ca, and Si, with preeminent phases encompassing FeO, $CaCO_3$, Ca_3SiO_5, Ca_2SiO_4, $Ca_2Fe_2O_5$, and SiO_2 [24]. The elevation in soil pH ostensibly ensues from the neutralizing influence of alkali compounds intrinsic to steel slag. Oxidative interactions of slag-borne substances with water yield -OH moieties, which subsequently engage in reactions with heavy metal cations within the soil milieu, precipitating hydroxides. In acidic soils replete with Cu^{2+}, a profusion of copper sulfide emerges via chemical precipitation reactions, culminating in the formation of $Cu(OH)_2$ [25,26]. Furthermore, Ca^{2+} within the steel slag engages in ion exchange with heavy metal ions in the soil, effectually securing heavy metals in situ [24]. It concurrently forms conglomerates with mineral colloids and organic constituents, thereby fostering a structural amelioration in acidic and heavy metal-afflicted soils [27].

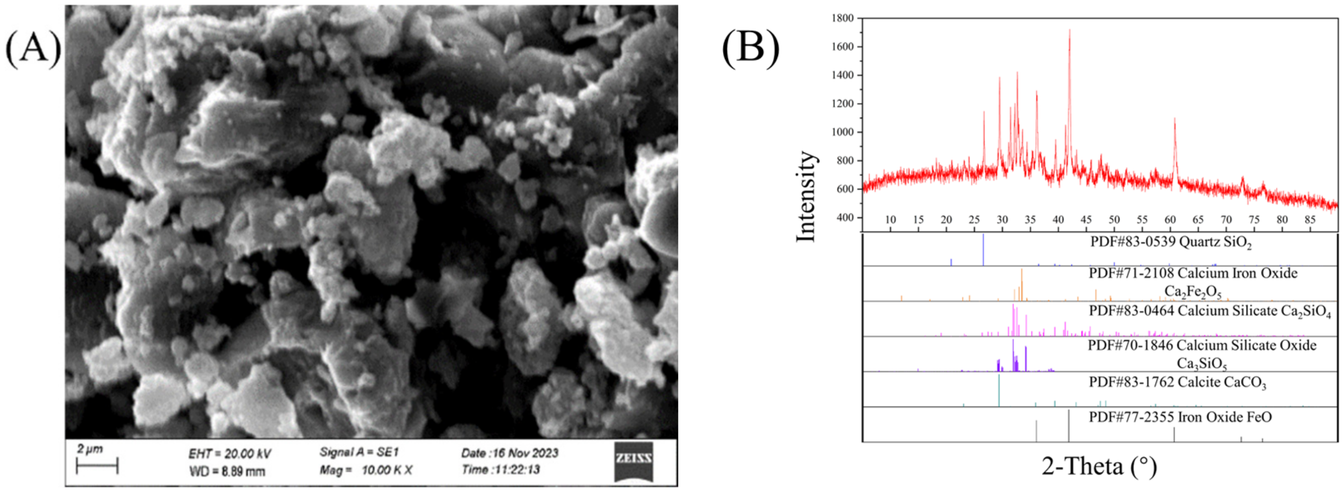

Figure 2. SEM images (**A**) and XRD patterns (**B**) of steel slag.

3.2. The Impact of Amendments on Soil Physicochemical Properties and the Bioavailability of Cu

The utilization of soil amendments has manifested improvements in soil physicochemical attributes to varying extents (Table 2). In contrast to the control (CK), the application of vermicompost and steel slag has exerted a salutary influence on soil pH. Notably, in the plot treated with vermicompost (L1), the pH has ascended from 3.78 to 5.17. In the plots ameliorated with steel slag (L2) and a composite of vermicompost and steel slag (L3), a more conspicuous augmentation in soil pH has been observed, reaching 6.76 and 6.86, respectively, from the baseline of 3.78. The escalation in soil pH is ascribed to the alkaline nature of steel slag, fostering the generation of hydroxides and the precipitation of carbonates [28]. Furthermore, the electrical conductivity (EC) within the soil across all treatment cohorts (L1, L2, and L3) has undergone diverse increments vis à vis CK, registering increments of 17.1%, 44.6%, and 34%, respectively. Concurrently, the cation exchange capacity (CEC) has undergone a notable surge, escalating from 17.2 cmol kg^{-1} to 30.6, 28.2, and 23.2 cmol kg^{-1}. Vermicompost and steel slag conduce to the augmentation of soil EC via the precipitation of soluble ions [29]. The heightened pH implies an accentuation of negative surface charges (OH^-), thereby instigating a heightened CEC [30]. Moreover, both the singular and concomitant application of amendments has signally elevated soil organic matter (SOM), particularly in the vermicompost-treated cohort (L1), where SOM has burgeoned by 147.9% compared to CK. The substantial augmentation in soil SOM is ostensibly attributable to the constituents bequeathed by vermicompost, with organic amendments serving as a direct fount for the amplification of SOM in the soil [31]. Intriguingly, post-amendment application, there was a marginal decrement in soil Eh. The inverse correlation between soil Eh and pH values substantiates this phenomenon [32].

Table 2. Basic physicochemical properties of soils under different treatments.

Treatments	pH	EC (mS cm^{-1})	SOM (g kg^{-1})	CEC (cmol kg^{-1})	Eh (mv)
CK	3.78 ± 0.45 c	858 ± 36.1 c	5.85 ± 0.05 d	17.2 ± 0.45 d	509 ± 6.06 a
L1	5.17 ± 0.17 b	1005 ± 73.4 bc	14.5 ± 0.27 a	30.6 ± 0.82 a	492 ± 3.38 b
L2	6.76 ± 0.04 a	1241 ± 47.2 a	9.90 ± 0.01 c	28.2 ± 0.26 b	417 ± 1.70 c
L3	6.86 ± 0.05 a	1150 ± 34.9 ab	12.5 ± 0.45 b	23.2 ± 0.51 c	403 ± 2.80 d

Note: different letters indicate obvious differences between treatment groups for the same index ($p < 0.05$). The data are mean ± SD, n = 3. EC, electrical conductivity; SOM, soil organic matter; CEC, cation exchange capacity; Eh, redox potential. CK: the control soil devoid of any amendment. L1: vermicompost amendment; L2: steel slag amendment; L3: a combination of vermicompost and steel slag amendment.

Due to the pronounced acidity inherent in the acidic soil derived from copper sulfide, characterized by a pH value of 3.78, the copper (Cu) within the soil manifests a heightened degree of bioavailability [33]. The application of vermicompost in isolation (L1), exclusive application of steel slag (L2), and the co-application of vermicompost and steel slag (L3) collectively yield a discernible reduction in both total and bioavailable Cu content within the soil matrix (Figure 3). Relative to the control group (CK), there is a notable decline in total Cu content of 43.2–44.7%, alongside an analogous decrease in bioavailable Cu content by 79.6–88.9%. Furthermore, the Cu bioavailability (Cu-BI) in soils subjected to L1, L2, and L3 applications exhibits a reduction of 64.2%, 80.3%, and 72.6%, respectively. These outcomes underscore the efficacy of steel slag application in enhancing Cu sequestration within the soil.

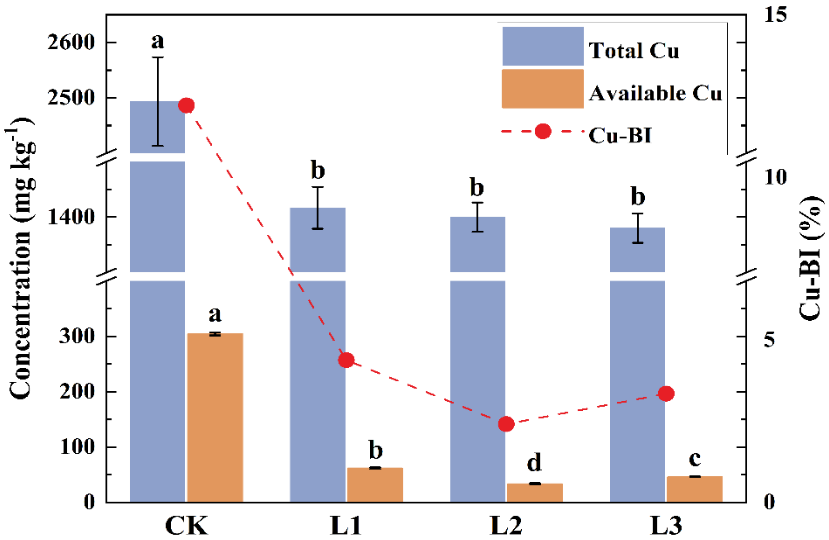

Figure 3. The total Cu concentration, available Cu concentration, and bioavailability of Cu in soil under different treatments. Note: The bars sharing the different letters suggest significant differences ($p < 0.05$). The data are mean ± SD, n = 3. CK: the control soil devoid of any amendment. L1: vermicompost amendment; L2: steel slag amendment; L3: a combination of vermicompost and steel slag amendment. The same as below.

The integration of SEM and XRD spectra unveils the substantial presence of alkaline oxides within the steel slag. This substantiates its robust acid-neutralizing capacity, thereby markedly elevating the concentration of hydroxide ions (OH^-) in the soil milieu. Consequently, this heightened OH^- concentration facilitates the precipitation of Cu^{2+} as insoluble $Cu(OH)_2$ [34]. Furthermore, SEM and FTIR analyses of vermicompost corroborate the abundance of organic functional groups. The undissociated functional groups form coordination bonds with liberated Cu^{2+} to promote the formation of less reactive metal complexes, thus significantly mitigating the toxicological impact of heavy metals [35].

3.3. Effects of Amendments on Biomass and Cu Bioaccumulation in L. perenne

In the preliminary experimental phase, it was discerned that the cultivation of black *L. perenne* in unmodified soil, particularly under the stringent conditions of acidic soil derived from copper sulfide, led to the curtailment of seed germination and the impediment of vegetative growth. The germination efficacy of black *L. perenne* was remarkably scant, culminating in comprehensive morbidity due to toxicity within a fortnight. Application of both vermicompost and steel slag, either independently or conjointly, evinced mitigating effects on the developmental trajectory of black *L. perenne*. The biomass of black *L. perenne* within the solitary vermicompost treatment cohort (L1) exhibited the nadir, while the amalgamated application of vermicompost and steel slag treatment group (L3) displayed the apogee in biomass (Figure 4A). Vermicompost and steel slag has been irrefutably substantiated to efficaciously enhance the physicochemical attributes of soil, endowing infertile soil with imperative nutrients, thereby catalyzing botanical proliferation and accentuating vegetative biomass [36–38]. The cumulative copper (Cu) accrual within the stems, leaves, and roots of black *L. perenne* adhered to the hierarchical sequence: the vermicompost treatment group (L1) surpassed the combined vermicompost and steel slag treatment group (L3), which in turn exceeded the steel slag treatment group (L2). In the vermicompost treatment group (L1), the cumulative Cu accumulation in the stems and roots of black *L. perenne* attained levels of 208.35 and 1412.05 mg kg^{-1}, respectively (Figure 4B). The attenuation in Cu accumulation observed in black *L. perenne* within the steel slag treatment group (L2) is attributable to the incorporation of steel slag, which precipitates an elevation in soil pH, consequently diminishing the bioavailability of Cu [39]. Within the vermicompost treatment group (L1), the substantial Cu accumulation in both the stems and roots of black *L. perenne* emanates from the diminished biomass, concomitant with a minor dilution effect. Additionally, the augmentation of soluble carbon in the soil via the application of vermicompost enhances Cu mobilization, facilitating its uptake by black *L. perenne* [26].

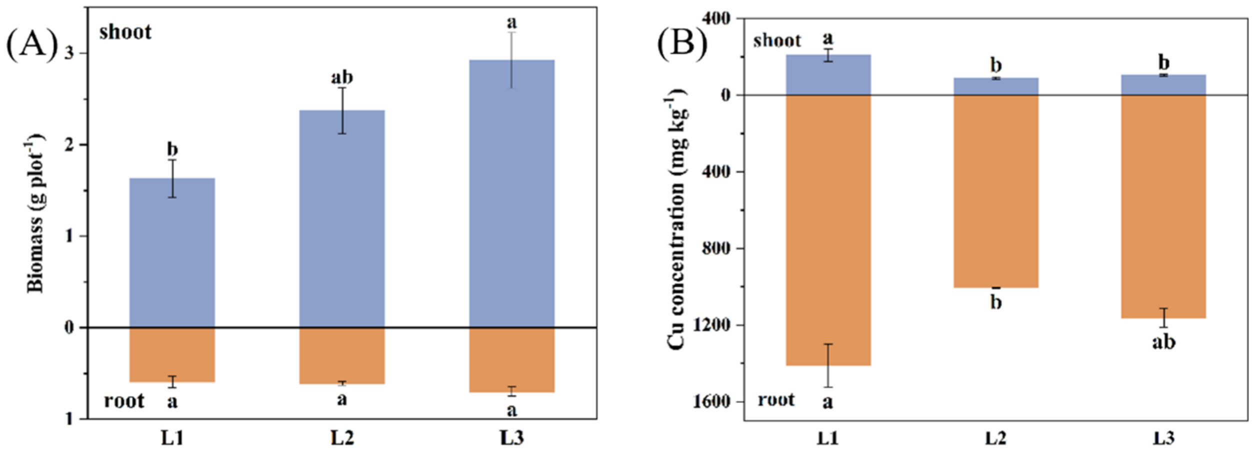

Figure 4. The biomass (**A**) and concentration of Cu (**B**) in *L. perenne* under different treatments. Note: the bars sharing the different letters suggest significant differences ($p < 0.05$).

3.4. Impact of Amendments on the Composition of Soil Bacterial Communities

Utilizing high-throughput sequencing methodologies, we analyzed the microbial community composition within soils subjected to diverse treatments. We derived results by employing a clustering threshold of 97% and normalizing to the minimum sample sequence count. The Venn diagram visually encapsulates the overlapping and distinct Operational Taxonomic Units (OTU) at the 97% similarity level across varied treatment modalities (Figure 5A). Common OTU shared among the control group (CK), the vermicompost treatment ensemble (L1), the steel slag treatment cohort (L2), and the confluence of vermicompost and steel slag treatment group (L3) were quantified at 65, with unique

OTU numbering 65, 42, 13, and 41, respectively. Relative to the control group, the addition of vermicompost and steel slag engendered a diminution in unique OTU within each treatment milieu, signifying an amelioration in the distinct bacterial taxa prevalent in acidic soil stemming from copper sulfide. Conversely, in comparison to the control group, all alternative treatment assemblages exhibited an augmentation in the aggregate number of bacterial OTU. Particularly noteworthy enhancements were discerned in the vermicompost treatment group (L1) and the combined vermicompost and steel slag treatment group (L3), showcasing an elevation of 126.04% and 119.53%, respectively.

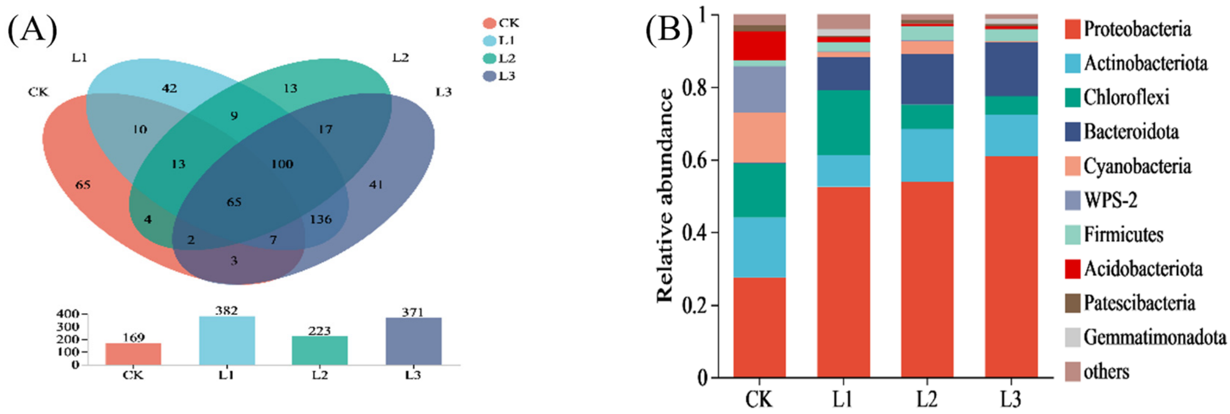

Figure 5. Venn diagram of soil bacteria at the OTU level (**A**) and the relative abundance of dominant bacterial communities at the phylum level (**B**) in soil under different treatments.

The taxonomic composition at the phylum level within distinct treatment cohorts exhibits congruity in predominant species but divergence in their relative abundances (Figure 5B). Broadly construed, the prevailing phyla in the bacterial consortium are *Proteobacteria* (27.48–60.94%), followed by *Actinobacteriota* (8.78–16.54%) and *Chloroflexi* (5.03–17.75%), collectively constituting over 60% of the bacterial assemblage. Antecedent investigations posit that Proteobacteria and *Chloroflexi* gradually ascend to the status of dominant species in soils contaminated with heavy metals, demonstrating formidable resilience to exigent environments [40,41]. In relation to the control group (CK), the augmentation of vermicompost and steel slag engenders an amplification in the prevalence of Proteobacteria, *Bacteroidota*, and Firmicutes while concurrently instigating a decrement in *Actinobacteriota*, WPS-2, and *Acidobacteriota*. Proteobacteria, characterized by heightened tolerance to pollutants, proficiently harness elevated concentrations of heavy metals and other deleterious substances as founts of energy and sustenance, thereby unveiling promising prospects for plant remediation and environmental safeguarding [42]. Furthermore, *Acidobacteriota* and *WPS-2*, ubiquitously distributed in desolate and exposed soil domains, undergo a marked attenuation in abundance within treatment groups subjected to amendments. This phenomenon is ostensibly attributed to the escalation in soil pH induced by the application of steel slag and vermicompost, effectually immobilizing Cu^{2+} [43–45].

3.5. The Relationship between Soil Physicochemical Properties and Bacterial Community

Envisaging the intricate interplay between soil physicochemical attributes and the intricate structure of bacterial communities, represented by the prevalence of ten prominent phyla, is achieved via the visualization derived from network analysis (Figure 6). Discerning from this analysis, a notably robust positive correlation emerges between Proteobacteria, *Bacteroidota*, and soil pH while concurrently revealing a pronounced inverse relationship with soil Eh and the aggregate Cu concentration. The soil's pH, recognized as a cardinal determinant, orchestrates a profound influence on the opulence of microbial communities therein [46]. The preeminent phylum, Proteobacteria, validated as an integral facilitator of plant flourishing, adept in the decomposition of soil macro-molecules and the facilitation of elemental cycling, surfaces as a key protagonist in this correlation [47]. *Acidobacteriota* manifests an unmistakable affirmative correlation with soil effective Cu concentration and an equally conspicuous negative association with soil EC. In stark contrast, Firmicutes, portraying a counteractive trend, exhibits a highly significant negative interrelation with soil effective Cu concentration and an inversely notable positive correlation with soil EC. This infers that the supplementation of vermicompost and steel slag serves to heighten the concentration of inorganic oxygen constituents such as Ca and Fe within the soil matrix, thereby exerting discernable effects on soil EC and effectively curtailing the bioavailability of Cu [48]. Concomitantly, soil SOM establishes a robustly affirmative correlation with *Gemmatimonadota*, juxtaposed against highly pronounced negative associations with *Actinobacteriota* and *Patescibacteria*. Scholarly investigations underscore the pivotal role of *Gemmatimonadota* in fostering vegetation recovery, with its abundance eliciting increasingly potent ameliorative effects on soil in tandem with rising soil fertility [49].

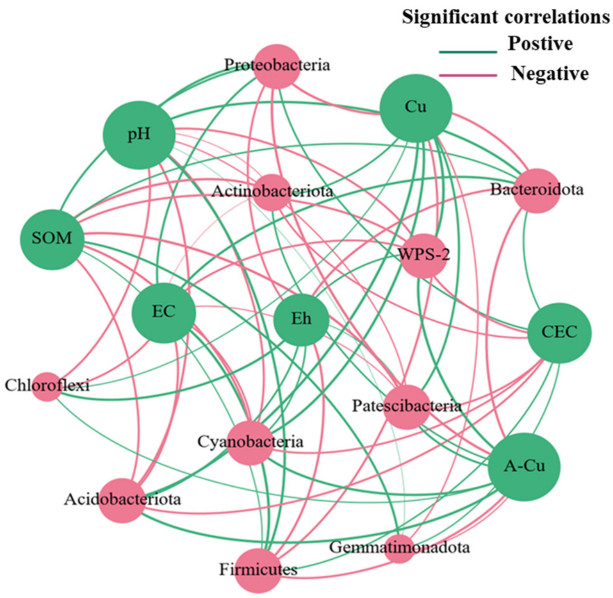

Figure 6. Co-occurrence network of soil physicochemical properties and dominant bacterial phyla in soil. Note: the size of the circles represents the number of connections in the correlation lines, and the thickness of the lines indicates the strength of the correlation, with thicker lines corresponding to larger absolute correlation coefficients and vice versa.

A thermal chart was constructed to appraise the intricate correlation existing between soil physicochemical attributes and the relative abundance of bacterial taxa, specifically the preeminent genera (Figure 7). The findings delineate a conspicuous positive correlation between *Phenylobacterium*, *Parasegetibacter*, *Micromonospora*, and soil EC, juxtaposed with a discernible negative correlation with the concentration of effective Cu within the soil matrix. Notably, robust positive correlation surfaces between soil SOM and the genera *Lysobacter* and *norank_f__norank_o__SBR1031*. Additionally, the genus Sphingomonas manifests a marked positive correlation with soil pH while concurrently exhibiting a markedly negative correlation with soil Eh and the aggregate concentration of total Cu. *Micromonospora* has been validated for its efficacy in fostering the growth of vegetation in soils laden with heavy metal contaminants [50]. The genera *Lysobacter* and *Sphingomonas*, owing to their pronounced adaptability to soil environments, exhibit notable potential in the remediation of soils subjected to heavy metal stress [51]. Ergo, the application of vermicompost and steel slag as amendments for the rectification of acidic soils hosting copper sulfide, begets alterations in the physicochemical milieu of the soil. Subsequently, this instigates a reshaping of the microbial community structure, ultimately culminating in an augmentation of soil quality.

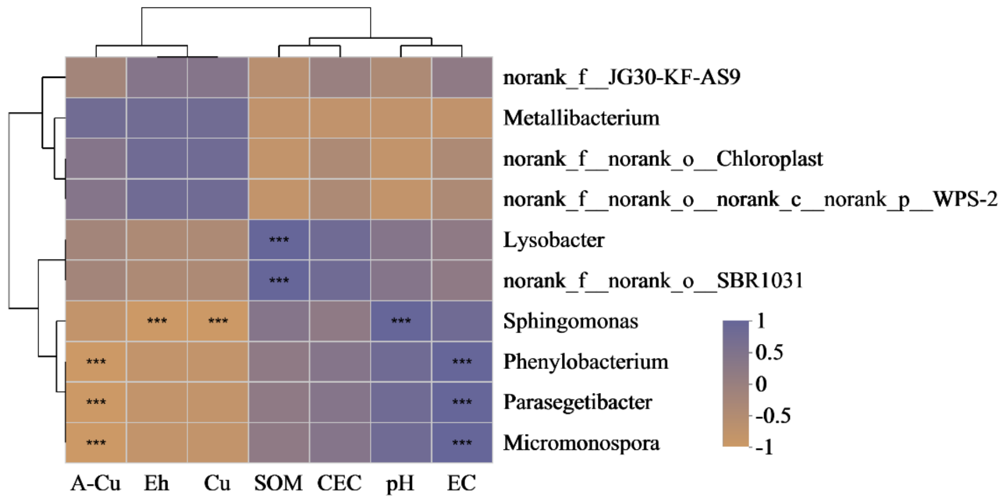

Figure 7. Heatmap of the correlation between soil physicochemical properties and the dominant bacterial phyla in soil. Note: *** $p \leq 0.001$.

4. Conclusions

This research endeavors to ameliorate acidic soils contaminated with copper sulfide via the application of a composite of *L. perenne* in conjunction with vermicompost and steel slag, either administered singularly or in tandem. The findings posit that *L. perenne* demonstrates notable resilience in adverse environments, rendering it a prospective phytoremediation candidate for soils afflicted by both acidity and copper pollutants. The solitary or combined application of vermicompost and steel slag substantively elevates soil parameters such as pH, electrical conductivity (EC), soil organic matter (SOM), and cation exchange capacity (CEC). This augmentation engenders an amplification in the abundance of bacterial communities concomitant with a diminution in soil redox potential (Eh), total copper content, and available copper content. It is noteworthy that the application of steel slag exhibits superior efficacy in immobilizing copper within the soil matrix, while vermicompost conduces to an augmentation in the diversity of soil bacterial communities. The ensuing alterations in soil physicochemical properties further precipitate a transformative impact on the composition of bacterial communities, and the joint application of steel slag and vermicompost serves to both enrich and stabilize the structure of soil bacterial communities. Thus, the synergistic application of vermicompost and steel slag in soils contaminated with heavy metals emerges as an environmentally conscientious and economically viable strategy for remediation.

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