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The Effects of Different Lead Pollution Levels on Soil Microbial Quantities and Metabolic Function with/without Salix integra Thunb. Planting

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Abstract: Background and Objectives: Salix integra Thunb., a fast-growing woody species, has been used in phytoremediation in recent years. It has the potential to accumulate high amounts of lead (Pb) in its growth, however, its effects on soil microbial community structure and function during its phytoreclamation processes are not well understood, especially at different pollution levels. Materials and Methods: In our study, we set unplanted and planted Salix integra in areas with four levels of Pb treatments (0, 500, 1000, and 1500 mg/kg). After six months of planting, the rhizospheric soil, bulk soil, and unplanted soil were collected. Soil properties and microbes participating in nitrogen and phosphorus cycling were measured, following standard methods. Microbial metabolic functions were assessed using a Biolog-ECO microplate. Results: The bacteria (nitrogen-fixing bacteria, ammonifying bacteria, inorganic phosphorus-solubilizing bacteria, and nitrosobacteria) all increased in the 500 mg/kg treatment and decreased in the 1500 mg/kg treatment compared with the 0 mg/kg treatment, especially in rhizospheric soil. The microbial metabolisms decreased along with the increase of Pb levels, with the exception of the rhizospheric soil with a 500 mg/kg treatment. The metabolic patterns were relative to the pollution levels. The utilization of carbohydrates was decreased, and of amino acids or fatty acids was increased, in the 500 mg/kg treatment, while the opposite occurred in the 1500 mg/kg treatment. The values of soil properties, microbial quantities, and metabolic activities were higher in rhizospheric than bulk soil, while the differences between bulk and unplanted soil were different among the different Pb treatments. The soil properties had little effect on the microbial quantities and metabolic activities. Conclusions: S. integra planting and Pb levels had an interactive effect on the microbial community. In general, S. integra planting promoted microbial quantities and metabolic activity in rhizospheric soil. Lower Pb pollution increased microbial quantities and promoted the utilization of amino acids or fatty acids, while higher Pb concentrations decreased microbial quantities and metabolic activities, and promoted the utilization of carbohydrates.

Keywords: phytoremediation; soil physicochemical properties; Biolog-ECO microplate; RDA analysis

1. Introduction  

Lead (Pb) is considered seriously harmful for human health because of its toxicity, persistence, bioaccumulation and biomagnification throughout the food chain [1], and high degree of accumulation in soil [2]. In the past five decades, more than 800,000 tons of Pb have been released into the
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environment globally, most of which has accumulated in soil and thus caused serious heavy metal pollution [3]. In addition, the problem of the soil environment in industrial and abandoned mining land is significant and serious. Therefore, it is a matter of great importance to remove Pb from soil in order to maintain a safe food chain and healthy environment. In general, physicochemical remediation technologies include soil excavation, soil cleaning, electrokinetic remediation, and extraction. However, these methods all have the problem of secondary pollution.

In the past three decades, phytoremediation technology has been proposed as a cost-effective and ecofriendly method for cleaning up polluted sites without secondary pollution [4]. Among the different techniques of phytoremediation, phytoextraction and phytostabilization are particularly relevant to alleviating metal pollution in soil [5]. Hyperaccumulators have also been strongly suggested as advantageous for phytoremediation. Hyperaccumulating plants exhibit foliar metal concentrations that are a minimum of 2–3 orders of magnitude higher than foliar concentrations found in other plants growing in normal soils, and at least one order of magnitude greater than the range in foliar concentrations of plants growing on metalliferous soils (established thresholds for hyperaccumulators growing in their natural habitats include 100 µg/g for Cd, Se, and Tl; 300 µg/g for Co, Cu, and Cr; 1000 µg/g for Ni, Pb, and As; 3000 µg/g for Zn; and 10,000 µg/g for Mn) [6]. However, the application of hyperaccumulators for phytoremediation is often limited by their slow growth rate and low biomass yield [7–9]. Trees, especially fast-growing woody species, such as Populus [10] and Robinia pseudoacacia L. [11], with their large biomass and deeper, more integrated root systems, have provided a unique means for the deep phytoremediation of soil [12,13].

Phytoremediation technology is based on the interactions of plants and microbes [14]. Root exudates stimulate the growth of specific bacterial and fungal populations, and change the microbial metabolic activity in rhizospheric soil [15–17]. In return, microbes modify the chemical composition of root exudates, the bioavailability of heavy metals, and soil physicochemical properties by their metabolic activities [18–20]. For example, plant colonization in mine tailings dramatically remodels the structure and function of the soil microbial community [19,21,22]. Zn pollution leads to decreased levels of basal respiration and ammonia-oxidizing bacteria, while Thlaspi caerulescens growth increases the values of substrate-induced respiration and total bacteria [23]. Similarly, planting of R. pseudoacacia also has a significant influence on bacterial communities in Pb/Zn contaminated soils [11]. Poplar phytomanagement regimes have led to decreased bioavailability of Zn and Cd, in concert with changes in the microbial communities [24]. Meanwhile, many fungi could alleviate heavy metal toxicity to plants and influence heavy metal accumulation and transportation [25–29]. Although advanced genetic editing of plants and bacteria may soon enhance phytoremediation even further [30], the current lack of reports about the impacts of metal contamination on microbial metabolic functions during phytoremediation makes their study of utmost importance.

In recent years, willow species, which can be grown intensively for use in energy production, have been suggested for use in the remediation of metal contaminated soils worldwide [9,31]. Much work has been conducted to evaluate the ability of Salix integra Thunb. to absorb heavy metals [32–36], especially Pb, and accumulation of up to 456 mg kg⁻¹ has been achieved in the shoots [37]. However, the effects of S. integra planting on the soil microbial community are not well understood, especially at different pollution levels, which limits our understanding of the phytoremediation processes of S. integra. Therefore, to better understand the phytoremediation mechanism of S. integra, we set unplanted and planted S. integra in areas with four levels of Pb treatments (0, 500, 1000, and 1500 mg/kg) in our study. We hypothesized that (1) S. integra planting would promote microbial metabolic activities and quantities of functional microbes in rhizosphere soil; (2) the effects of different Pb pollution levels on microbial metabolic activities and quantities of functional microbes would be different; and (3) microbes and microbial metabolic functions would be affected by the soil properties.
2. Materials and Methods

2.1. Study Site and Plant Material

The study site was located in the experimental station of Hebei Agricultural University, with a temperate climate (Baoding city, Hebei province, China. 38°45′21″ N, 115°24′37″ E). The mean annual temperature is approximately 13.0 °C and the annual precipitation is about 532 mm. The soil is a typical meadow cinnamon soil (a transition type from cinnamon soil to meadow soil). _Salix integra_ is a shrub of the family Salicaceae. One year cutting seedlings of _S. integra_ with the same growth vigor were selected for use in the study.

2.2. Experimental Set Up

The experiment was carried out by excavating four square plots below the ground level. The size of each plot was 4 m (length) × 4 m (width) × 0.6 m (depth). Perfluorinated ethylene-propylene copolymer (PEP) plastic sheets were used to line the bottom of the four plots, which played an anti-seepage role. Finally, the soils were backfilled correspondingly. This method avoids the disadvantages of pot experiments, in which soil compaction can occur easily and thereby limit plant growth. A rain shed with a transparent plastic sheet was built above the study area to control the experiment.

In our study, four treatment regimens of Pb were conducted in each of the plots. They were 0 mg/kg (CK), light pollution 500 mg/kg (LT), medium pollution 1000 mg/kg (MT), and high pollution 1500 mg/kg (HT), respectively. Pb was injected into the soil as Pb(NO$_3$)$_2$ solution in June 2017. The solution volume was calculated by the water holding capacity. The soils were subjected to aging and equilibration under natural conditions for three months. During the aging period, the soil moisture was maintained at approximately 75% of the maximum water holding capacity. Meanwhile, the soils were ploughed frequently. In each treatment, we set the unplanted area and planted _S. integra_ area at the same time. The different treatment areas were separated by a PVC sheet. The layout of the study site is shown in Figure 1. The initial soil properties were as follows: organic carbon (2780 mg/kg), total nitrogen (240 mg/kg), available nitrogen (22.28 mg/kg), total phosphorus (510 mg/kg), available phosphorus (10.54 mg/kg), total potassium (8080 mg/kg), available potassium (278.4 mg/kg), cation exchange capacity (8.46 cmol/kg), and pH (8.14).

One year cutting seedlings were planted in May 2017. Eight trees were planted in each treatment. The control and treatment groups were all under the same management conditions (watering, loosening, weeding, etc.) during the study period.

![Figure 1](image-url)  
*Figure 1. The layout of the study site. The plots numbers (1, 2, 3, and 4).*

2.3. Soil Collection

After six months growth, the rhizospheric soil, bulk soil, and unplanted soil were collected in November, in three repetitions under each treatment (sampled in plots 1, 2, and 4). The following method was utilized for rhizospheric soil: the roots were recovered from the soil cores (the diameter
10 cm, and the depth 20–30 cm), then gently shaken to remove any loosely adhering soil, and after that placed in a sterile plastic bag and shaken dramatically. The soils from five plants were pooled together, thus giving a mixed sample of rhizospheric soil as one replication. The bulk soils and unplanted soils were all obtained by soil-drilling. The bulk soils were collected around the tree, and the unplanted soils were randomly collected from the unplanted area. Soils from five random spots were mixed as one replication. Each replication sample was divided into five parts for the measurement of soil nutrients, pH, cation exchange capacity, quantities of soil culturable microbes relative to nitrogen and phosphorus cycling, and community level physiological profiling (CLPP). After removal of crop residues, the soil samples used for measuring culturable microbes and CLPP were transported to the laboratory immediately in sterilized plastic and stored at 4 °C. The tools were cleaned and disinfected between different treatments when sampling the soil.

2.4. Analysis Methods

2.4.1. Soil Properties Analysis

The soils were air dried and sieved to a diameter of < 2 mm. The organic carbon (SOC) was measured by the potassium dichromate heating method after the soils were digested with H₂SO₄. Available nitrogen (AN) was analyzed by the alkali diffusion method. Available phosphorus (AP) was analyzed by Mo–Sb colorimetry (using a Vis spectrophotometer, 722S, Jinhua China) after the soil was extracted with sodium bicarbonate. Available potassium (AK) was analyzed with atomic absorption spectrophotometry (AA-680, SHIMADZU) after the soil was extracted with CH₃COOH. The pH value was measured in deionized water (1:2.5 m/V) using a pH meter. The cation exchange capacity (CEC) was measured by the method of barium chloride–sulfuric acid exchange [38].

2.4.2. Quantities of Soil Culturable Microbes

The quantities of nitrogen-fixing bacteria (NFB) and inorganic phosphorus-solubilizing bacteria (IPB) were enumerated using the dilution plate method [39]. In brief, 10 g soil (dry weight equivalent) was suspended in 90 mL sterile water and stirred vigorously for 30 min. The serial dilution was plated onto different media (one sample made three replications). 1 L medium of NFB contained glucose 10.0 g, agar 18 g, K₂HPO₄ 0.5 g, MgSO₄·7H₂O 0.2 g, NaCl 0.2 g, MnSO₄·4H₂O (10 g/L) 2 drops, and FeCl₃·6H₂O (10 g/L) 2 drops. After sterilization, the initial pH was set to 7.0 (using 0.1 mol/L NaOH). Next, 5 mL Congo red solution was added. 1 L medium of IPB contained glucose 10.0 g, agar 20 g, yeast extract 0.5 g, CaCl₂ 0.1 g, and MgSO₄·7H₂O 0.3 g. After sterilization, CaCl₂ solution (100 g/kg, 10 mL) and K₂HPO₄ solution (100 g/kg, 1 mL) were added in every 50 mL media when using the medium. The initial pH was also set to 7.0 (using 0.1 mol/L NaOH). Bacterial quantities were counted as colony-forming units (CFU).

Nitrosobacteria (NB) and ammonifying bacteria (AMB) were measured by the most probable number (MPN). Preparations of the culture media were followed by experimental instruction [40]. 1 L medium of NB contained (NH₄)₂SO₄ 2.0 g, K₂HPO₄ 0.75 g, NaH₂PO₄·7H₂O 0.25 g, MnSO₄·4H₂O 0.01 g, MgSO₄·7H₂O 0.03 g, and CaCO₃ 5.0 g. The initial pH was set to 7.2. The detection reagent was Griess reagent. 1 L medium of AMB contained peptone 5.0 g, K₂HPO₄ 0.5 g, KH₂PO₄ 0.5 g, and MgSO₄·7H₂O 0.5 g. The detection reagent was Nesster’s reagent. The bacterial quantities were calculated by MPN table.

2.4.3. Community Level Physiological Profiling

CLPP is a useful tool for assessing microbial community metabolic function and functional diversity in both terrestrial and aquatic environments [41–43]. CLPP, also known as carbon source utilization patterns of soil microbial communities, is assessed using a Biolog-ECO micro-plate (Category NO. 1506 U.S.A.). One plate, containing 31 carbon sources and one blank (in triplicate), was used in each sample. The soil was sifted to remove impurities. A subsample of 10 g (dry weight equivalent) of each
soil sample was suspended in 90 mL of 0.1% sterile sodium chloride (NaCl) solution, and then shaken for 30 min (200 r/min) at room temperature. The supernatant was diluted $10^3$ times, and the diluted soil suspension (150 µL) was added to each well of a Biolog-ECO micro-plate. The micro-plate was incubated at 25 °C in darkness. Optical density (OD) was measured with SpectraMax384 (Molecular Devices, U.S.A.) at 590 nm (color and turbidity) and 750 nm (turbidity) wavelengths every 24 h until no more growth in the OD value could be observed. The procedure lasted up to eight days to ensure the carbon utilization saturation phase was reached in all samples [44].

For all samples, absorbance readings at 144 h were identified as the time point for further CLPP data analysis. Data were analyzed to determine metabolic diversity indices, according to Sofo’s study [45]. The indices examined were as follows: average well color development (AWCD) that were utilized to reflect microbial metabolic activity [46], richness index (S), Shannon’s diversity index ($H'$), Shannon’s evenness index (E), Simpson’s diversity index (D), McIntosh’s diversity index (U), and McIntosh’s evenness index (Z).

2.5. Statistical Analyses

Each data point represented the results of triplicate sample analysis, and each value was expressed as the mean ± standard error. A two-way ANOVA was applied to compare the impact of the two factors (Pb concentration and different sources of soil) on significant differences of indices among different treatments. The normality of the analyzed features was checked with the Shapiro–Wilk test. Depending on the distribution of the estimated parameters, either the least significant difference (LSD) in the parameter test or the Kruskal–Wallis test in the non-parameter test was used to check for significant differences among the treatments. In all analyses, differences were considered significant at a $p$ value < 0.05. The above statistics were performed with the package SPSS Statistics 19.0 (IBM, Armonk, NY, America).

Principle component analysis (PCA) was performed using the data matrix (11 samples and 31 variables) of AWCD normalized carbon source utilization patterns data to further assess the differences among different treatments. Redundancy analysis (RDA) was conducted to test the relationship of soil properties and microbial communities. The analyses of PCA and RDA were all performed by the software CANOCO (Windows version 4.5) (Biometris-Plant research international, Wage-ningen, the Netherlands).

3. Results

3.1. Soil Physicochemical Properties

The interactive effect (PbC × DS) of Pb concentration effect (PbC) and different sources of soil (DS) was significant on AN, AP, AK, and CEC, but not on pH (Figure 2). The values of CEC were increased in LT and MT and decreased in HT, especially in rhizosphere (Figure 2d). The contents of AP and AK were strongly inhibited in Pb treatments no matter whether in rhizosphere, bulk, or unplanted soil. The inhibitory effects were higher with the increase of Pb concentration (Figure 2b,c), while the AN was promoted when compared with CK (Figure 2a) and was highest in MT.

The effects of $S$. integra planting varied based on different Pb treatments. In general, the values of AK, AN, AP, and CEC were higher in rhizosphere than bulk soil, regardless of CK, LT, MT, or HT. However, the trends were complex when compared with unplanted soil. For example, the AK of unplanted soil was lowest in MT and HT, while it was highest in CK and LT. The effects of both Pb pollution and $S$. integra planting on pH were not significant.
Figure 2. Soil properties of available nitrogen (a), available phosphorus (b), available potassium (c), and cation exchange capacity (d). No pollution (CK), 0 mg/kg; light pollution (LT), 500 mg/kg; medium pollution (MT), 1000 mg/kg; high pollution (HT), 1500 mg/kg; R, rhizospheric soil; B, bulk soil; U, unplanted soil. PbC, Pb concentration effect; DS, different sources of soil; PbC × DS, the interactive effect of Pb concentration and different sources of soil. Different letters indicate that values differ significantly at \( p < 0.05 \). The lowercase letters represent the global comparison and uppercase letters represent the intra-soil type comparisons. Values represent mean ± SD (\( n = 3 \)).

3.2. The Quantities of Soil Culturable Microbes

The interactive effect of PbC × DS was significant on the four kinds of bacteria (Figure 3a–d). Compared with the CK, the quantities of IPB were increased in LT and decreased in MT and HT, whether in rhizosphere, bulk, or unplanted soil (Figure 3b). The AMB and NFB showed a similar trend only in rhizosphere soil, and they increased in LT and decreased in MT and HT when compared with the CK. However, the AMB decreased in bulk soil of Pb pollution treatments and increased in unplanted soil of HT, and the NFB decreased only in bulk soil of HT and increased in unplanted soil of LT compared with the CK (Figure 3a and d). The quantities of NB were increased in the MT and LT of rhizosphere soil, and decreased only in the unplanted soil of HT (Figure 3c). The four kinds of bacteria were all highest in rhizosphere, while the difference between bulk and unplanted soils varied among the different treatments.
3.3. Metabolic Activity of Microbial Communities

The dynamic patterns of AWCD values are shown in Figure 4a–e. The AWCD values were relatively small with 24 h of incubation, which indicates that the carbon sources were not utilized during that period. After that, the AWCD value increased gradually as time went on. The AWCD values changed with time in a similar trend among the different treatments. *S. integra* planting promoted the metabolic activities of microbial communities, which were all higher in rhizospheric soil, and the differences increased with the increase of Pb concentrations from LT to HT. The AWCD values of unplanted soil were higher than those of bulk soil in the LT. However, the differences between bulk and unplanted soil were small in MT and HT.

The carbon source utilization rates were basically stable at 144 h, so the AWCD values for 144 h were used for further analysis (Figure 4e). There was no significant difference between LT and CK in rhizospheric soil, and LT and MT in bulk soil. The others were reduced with increased Pb concentrations.
3.4. Utilization of Specific Carbon Sources

Thirty one carbon sources in Biolog-ECO plates were divided into four categories, according to the microbial metabolic pathway of three major nutrients. The categories were carbohydrates and their derivatives (CG, 12 kinds), amino acids and their derivatives (AG, 6 kinds), fatty acids and lipids (FG, 5 kinds), and metabolic mediates and secondary metabolites (MG, 8 kinds) [47].

Pb concentrations, carbon sources, and their interactive effect significantly affected the AWCD (Figure 5a–c). The utilization of specific carbon source types changed with different Pb levels, as compared with the CK. The CG, AG, and MG showed no significant differences between CK and LT, but four categories were all decreased in HT in rhizospheric soil (Figure 5a). The four categories were all decreased as compared with the CK in bulk soil, and the adverse effects were higher with increased Pb concentrations (Figure 5b). The highest utilization of specific carbon sources were FG in CK and CG in HT, while the FG, CG, and AG showed no significant differences in LT and MT in rhizospheric soil. FG was highest in LT and MT, and FG, CG and AG showed no significant differences in CK and
HT in bulk soils. The FG and CG were highest in LT, CG was highest in HT, and FG, CG and AG showed no significant differences in MT in unplanted soil (Figure 5c). However, MG was lowest in all the treatments.

Furthermore, we compared the relative utilization percentage of the specific carbon source in each treatment (Figure 5d). We found that the CG utilization was decreased both in rhizospheric soil and bulk soil, while AG was increased in rhizospheric soil, and FG was increased in bulk soil when compared LT with CK. In contrast, the CG utilization was increased both in rhizospheric soil and bulk soil, when MT and HT were compared with CK. Decreases of AG and MG were found, respectively, in rhizospheric and bulk soil under both MT and HT. The CG utilization was increased with the increase of the Pb concentrations in unplanted soil.

3.5. Microbial Community Metabolic Profiles

The carbon source utilization patterns (CSUPs) of microbial communities were visualized using principal component analysis (PCA). According to the results (Figure 6), the variance contribution rate of the first principal component (PC1) was 42.5%, and the second principal component (PC2) was 19.0%. All the samples were distributed along the positive direction on the PC1 axis.

The microbial CSUPs formed four groups. From right to left, the first group contained the rhizosphere of CK, LT, and MT and the bulk soil of CK, while the rhizosphere of HT was close to
the second group, which included the bulk and unplanted soils of LT. The third and fourth groups contained the bulk and unplanted soils of MT and HT, respectively. This result indicates that the soil microbial community had a unique model of carbon utilization in rhizosphere soil, while the bulk and unplanted soils were distinctive in relation to the contamination levels.

**Figure 6.** Principal component analysis based carbon source utilization patterns of microbial communities. PC1, the first principal component; PC2, the second principal component; CK, 0 mg/kg; LT, 500 mg/kg; MT, 1000 mg/kg; HT, 1500 mg/kg; R, rhizospheric soil; B, bulk soil; U, unplanted soil. The data matrix included 11 samples (each was the mean of 3 repetitions) and 31 variables.

### 3.6. Diversity Indices of Soil Microbial Communities

The interactive effect of PbC $\times$ DS was significant on $S$, $U$, and $Z$ (Figure 7a,c,d). LT increased the values of $S$, and MT and HT decreased the values of $U$ in rhizospheric soil as compared with CK. The values of $S$ and $U$ were all decreased in bulk soil, when compared with CK. The adverse effects were higher with increasing Pb concentration in unplanted soil, no matter the values of $S$, $U$, or $Z$. The effects of *S. integra* planting promoted the values of $S$, $U$, and $E$ to be, in general, higher in rhizospheric soil than bulk soil. However, the trends were complex when compared with unplanted soil.

When the interactive effect of PbC $\times$ DS was not significant, we just compared the significant main effect, for example $E$ and $H'$ (Figure 7b,e). The values of $E$ and $H'$ in rhizospheric soil were higher than in bulk and unplanted soils. Neither PbC nor DS had a significant effect on the values of D.

**Figure 7.** Cont.
Figure 7. Microbial communities diversity indices of richness index (S, a), Shannon’s evenness index (E, b), McIntosh’s diversity index (U, c), McIntosh’s evenness index (Z, d) and Shannon’s diversity index (H’, e) based on 144h data from Biolog analysis under different treatments. CK, 0 mg/kg; LT, 500 mg/kg; MT, 1000 mg/kg; HT, 1500 mg/kg; R, rhizospheric soil; B, bulk soil; U, unplanted soil. PbC, Pb concentration effect; DS, different sources of soil; PbC × DS, the interactive effect of Pb concentration and different sources of soil. Different letters indicate that values differ significantly at p < 0.05. The lowercase letters represent the global comparison and uppercase letters represent the intra-soil type comparisons. Values represent mean ± SD (n = 3).

3.7. Relationships of Microbial Quantities, Metabolic Profiles and Soil Chemical Properties

The relationships of soil properties relative to microbial quantities and metabolic profiles were examined by RDA analysis (Figure 8a,b). Eigenvalues of RDA showed that axes 1 and 2 explained 44.5% and 14.5% of the variance of the microbial quantities data, respectively (Figure 8a). CEC values were significant (F = 4.169, p = 0.012) in explaining the variations of microbial quantities, and could explain up to 49.6% of the variation. The contributions of other factors to the observed variation were as follows: AN (22.5%), AK (10.6%), pH (6.4%), and AP (10.8%). NB had a significant negative correlation with AP (p = 0.045), and had a significant positive correlation with AN (p = 0.015). IPB and NFB had significant positive correlations with CEC (p = 0.015) and AK (p = 0.033), respectively.

As to the metabolic activity, the eigenvalues of RDA showed that RDA1 and RDA2 only explained 15.6% and 2% of the varieties of soil microbial carbon utilization rate profiles (Figure 8b). The soil properties made no significant contribution to the variation of metabolic activity. CG was negatively correlated with all factors, and had significant correlation with AK (p = 0.007) and CEC (p = 0.023).
4. Discussion

4.1. Effects of *S. integra* Planting and Pb Pollution on Soil Properties and Microbial Quantities

The four kinds of bacteria were all increased in LT and decreased in HT compared with CK, especially in rhizospheric soil. A higher Pb concentration suppressed microbial growth, such that certain sensitive taxa were extinguished by heavy metal poisoning. For example, the relative abundance of *Firmicutes* decreased from 88.5% (CK) to 12.1% (Pb pollution treatment) during phytoremediation by *Trifolium repens* L. [48]. Zhu et al. [48] also suggested heavy metal contamination could play a more important role in shaping bacterial diversity. The bacterial growth rate and biomass in soils was found to consistently diminish along the Cd gradient, regardless of planting *Sedum alfredii* [49]. Similarly, Golebiewski et al. [50] found that species richness, diversity, and bacterial communities in soils with low levels of Pb contamination were higher than in highly polluted soils. This study found that microbial quantities were increased in lower Pb concentrations, which conflicts with former studies. We inferred that the root exudates were stimulated by a lower Pb concentration, which attracted more bacteria in return. Our study concerned the culturable bacteria only, and because of the limitation of the cultivation approach for enumeration [51], it is not guaranteed that these results can be generalized to non-cultivable microbes.

In general, AP and AK were decreased with the increase of Pb concentrations, while AN was increased. There are two reasons for this, with the first being that the chemical effect of adding Pb had significant effects on ion exchange in the soil. For example, Pb$^{2+}$ reacted with PO$_4^{3-}$ to form Pb$_3$(PO$_4$)$_2$ (insoluble matter), especially in alkaline soil, which led to the decrease of AP [52]. Another reason may be that heavy metal pollution induced changes in the microbial community structure [53–55] and thereby affected nutrient cycling, especially available phosphorus and potassium [50,56–59]. However, the trends of microbial quantities and nutrients in response to Pb concentration were different in our study. The reason for this may be that the nutrient cycles required microbes with different functions, for example, the microbes involved in the nitrogen cycle, including nitrogen-fixing bacteria, ammonifying bacteria and nitrifying bacteria. Consistent with the RDA analysis, the explanatory variables of AN, AP, and AK had a relatively small impact on microbial quantities. The reason for this may be that we just measured four types of microbes, which were only a small proportion of the microbes involved in N and P cycling. At the same time, adding Pb$^{2+}$ could change other soil properties.
including redox potential and cation/anion exchange capacity [60]. It partly removed cations (Na\(^+\), K\(^+\), Ca\(^{2+}\), etc.) from the soil solution that led to the increase of CEC [60,61]. However, the CEC was decreased in the HT, which may be related to the balance of soil properties to the higher Pb pollution.

In general, \(S.\) \(integra\) planting increased the values of soil properties and microbial quantities to a greater degree in rhizosphere than bulk soil, across all Pb treatments. However, the trends were complex when compared with unplanted soil. These were consistent with previous studies’ indications that microbial biomass clearly increased in rhizospheric soils compared to bulk soil [16,62–64]. Similarly, \(T.\) \(caerulescens\) growth increased microbial respiration rates in the Zn- and Cd-contaminated soil [23]. Planting of \(R.\) \(pseudoacacia\) and \(Populus\) also had a significant influence on the rhizospheric bacterial community structure in heavy metal contaminated soils [11]. These findings could be partly explained by the reduction of metal concentration in the rhizospheric soil due to plant uptake. Yang et al. [62] reported that phytoextraction by \(Sedum\) \(alfredii\) significantly reduced the available and total Cd of 0–2 and 2–4 mm soils. In addition, there were more root exudates in the rhizosphere environment, which may lead to higher microbial populations and stimulate the growth of specific bacterial and fungal populations [15–17]. Furthermore, soil organic carbon sources excreted by roots were reported to be vital in determining rhizospheric soil microbial compositions [65]. The values of soil properties that were increased also contributed to the increase of root exudates and microbial quantities in rhizospheric soil. In contrast, Epelde et al. [23] reported lower values of total N and extractable K\(^+\) observed in planted versus unplanted soils because of plant uptake. The root exudates, such as carboxyl, phenolic, hydroxyl, and carbonyl groups, led to negative charges on the soil surface, and cations could be partly removed from the soil solution, thereby increasing the CEC in the rhizospheric soil [60,66].

The difference between bulk soil and unplanted soil seen with \(S.\) \(integra\) planting not only had direct effects on rhizospheric soil, but also had indirect effects on bulk soil. As in the earlier studies, the effects of the rhizosphere should not be limited to specific volumes [62,67]. For instance, the rhizosphere effects of \(Sedum\) \(alfredii\) on the microbial community structure and metabolic function were not limited to the root surface, notwithstanding that the effect decreased as the distances increased [62]. Similarly, the interactive effects of pioneer plants and harsh soil environmental conditions remodel the specific bacterial communities, both in rhizosphere and bulk soil, in mine tailings [19]. The reasons for this may be that the root exudates could be transported from rhizospheric soil to bulk soil by active transport (animal and fungal mycelium) or passive transport (concentration difference and water action) [68].

4.2. Effects of \(S.\) \(integra\) Planting and Pb on Microbial Metabolic Profiles

\(S.\) \(integra\) planting promoted microbial metabolic activities and diversity in rhizospheric soils, while Pb pollution suppressed microbial metabolic activity and diversity. The inhibitory effect was increased with increasing Pb concentration, with the exception of LT in rhizospheric soil. This finding was consistent with the results regarding microbial quantities in our study. Many researches have indicated that excessive metals in soil are toxic to microbes, and that the ecological functions performed by microbes could be seriously inhibited [69–71].

The soil microbial community showed a unique pattern of carbon utilization in rhizospheric soil samples. Bulk and unplanted soil samples showed carbon utilization relative to the contamination levels. The microbial community increased the utilization of AG and MG in rhizospheric soil that underwent low Pb treatment, and increased the utilization of CG under higher Pb pollution. Therefore, we inferred that the dominant species of the microbial community changed. When subjected to heavy metal stress, some microbial populations become extinct, while certain resistant community members survive and form the basis of a new community [72]. Plant–microbes–environment balance and interact with each other during phytoremediation. This process is very complex.

Some researchers have shown that the composition and quantities of root exudates change a lot when plants respond to heavy metal stress [60,73,74]. Plants can be selected for beneficial microbial
communities in their rhizosphere by releasing certain metabolites targeting specific microbes [75]. AG and CG were the major components of root secretion. Amino acids and organic acids, which are released from plant roots, have been shown to enhance phytoextraction of heavy metals by increasing their bioavailability [75–78]. Alanine and proline activated Pb and increased the Pb uptake ability of a Pb-accumulating ecotype of Sedum alfredii [79]. The literature about the effect of carbohydrates is relatively sparse, however, it is suggested that they could immobilize Pb$^{2+}$ and Cu$^{2+}$ and prevent them entering into the plant [80]. Dissolved organic matter, which is released from the rhizosphere of S. alfredii, could markedly decrease Zn and Cd adsorption and enhance their mobility by forming soluble organic matter–metal complexes [81]. Of course, detailed information about the components of root exudates from S. integra require further study to reveal the mechanism for regulating microbial metabolism activity.

Environment factors only explained 14.8% of the variance of soil microbial carbon utilization rate profiles. Only AK and CEC were significantly negatively correlated with CG, which further shows that the environmental factors determined by us are not the main reason for the metabolic differences of the microbial community. Therefore, root exudates and other factors, including heavy metal content, should be taken into consideration.

5. Conclusions

S. integra planting increased the microbial quantities and metabolism activity in rhizospheric soil. The effect of S. integra growth was observed not only in the rhizosphere, but also in bulk soil, although the range of influence requires further study. Microbial quantities and metabolic patterns were relative to the Pb pollution levels: a low concentration increased the microbial quantities and a high concentration decreased it, to different degrees. The microbial metabolisms were decreased with the increase of Pb levels, with the exception of the rhizospheric soil of LT. The utilization of CG was decreased, and of AG and FG was increased in the low Pb treatment, while the trends were opposite to this in the high concentration condition. Therefore, we believe that S. integra develops its own strategy to survive in different levels of polluted soil by changing root exudates, and in doing so further regulates the microorganism community. In return, microbes activate different defense mechanisms to eliminate Pb stress. Therefore, advancing the current understanding of the interaction of the root–microbial community will be essential in the future, for example by conducting metabonomic research to analyze root exudates and microbial metabolism by Gas Chromatography-Mass Spectrometer (GC-MS) or nuclear magnetic resonance (NMR).

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