EFFECTS OF INTERCROPPED *LEERSIA HEXANDRA* AND CARBON SOURCE AMENDMENT ON NUTRIENT AND MICROBIAL CHARACTERISTICS IN CHROMIUM POLLUTED SOIL

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Abstract. To explore the safe utilization of Cr-contaminated soil, in a pot experiment, the impacts of applying carbon source amendments, sole cropping of *Leersia hexandra*, and combined treatments on soil mechanical composition, nutrient content, enzyme activity, and microbial quantity were investigated. The results revealed that compared to the control, all treatments were able to alter the mechanical composition of Cr-contaminated soil, organic matter, and the content of available nitrogen, phosphorus, and potassium in soil. They also increased the activities of urease, phosphatase, hydrogen peroxidase, and sucrase enzymes, as well as the populations of bacteria, actinomycetes, and fungi. Among the treatments, those with the most significant effects on increasing available potassium content and soil fungi quantity were BS (biochar + sugarcane bagasse) and BSL (biochar + intercropping + sugarcane bagasse), while BL (biochar + intercropping) had the most significant impact on all parameters compared to their respective sole treatments, demonstrating a pronounced synergistic effect. The combination of biochar and intercropping with hyperaccumulating plants can effectively leverage the advantages of amendments and intercropping in improving the nutrient status of polluted soil, thus providing valuable insights for the safe utilization of Cr-contaminated soil.
Keywords: *biochar, soil amendments, enzyme activity, microbial biomass, heavy metal remediation, soil fertility*

Abbreviations: OM, organic matter; TOC, organic carbon; AN, alkali-hydrolyzable nitrogen; AP, available phosphorus; FTIR, Fourier transform infrared spectrometer; XRD, X-ray diffraction

Introduction

Soil quality has the most direct, profound and long-term impact on the sustainability of agriculture. Improving soil quality is the key to achieve the United Nations sustainable development goals (Keesstra et al., 2016). In recent years, with the rapid development of industry and agriculture, the problem of heavy metal pollution in soil is becoming more and more prominent, resulting in the decline of soil environmental quality, which has potential harm to human health and agricultural productivity (Mishra et al., 2019). Chromium (Cr) is considered as one of the most harmful elements, which can lead to soil degradation, endanger the yield and quality of crops, and pose a threat to human health through the food chain (Zeng et al., 2011).

At present, immobilization in situ remediation technology and phytoremediation technology are two major technologies widely used responding to chromium-contaminated soil (Oseni et al., 2020). Adding soil amendments is considered to be an economic, effective and eco-friendly remediation way. The remediation materials for

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heavy metal passivation in soil are low in cost, significant in efficiency, environmentally friendly, and suitable for large areas of contaminated farmland (Liu et al., 2018). Biochar is a new and efficient soil improvement material emerging in recent years. It is rich in carbon, which can increase the carbon sink of farmland soil and improve soil fertility. Moreover, its porosity and specific surface area are large, and it has a good adsorption performance, which can provide an attachment carrier for microorganisms, as well as a carbon source and nutrient source for the growth of microorganisms. It has a good effect on the passivation of soil heavy metal pollution (Guo et al., 2020). Bagasse is an easily obtained agricultural waste, which has been developed as a low-cost adsorbent with high performance. The remediation mechanisms for heavy metal ions mainly include electrostatic attraction, chemical adsorption, and ion exchange (Raj et al., 2022). It has been studied that the surface modification of bagasse effectively improves the adsorption efficiency of chromium (Garg et al., 2005). By adding these two materials to the soil, and through a series of physical and chemical reactions such as adsorption, precipitation, complexation and ion exchange, the activity of ions can be effectively decreased to reduce the environmental risk of heavy metals in the soil.

Phytoremediation is an environmentally friendly, low-cost and simple treatment technology with good development prospects. Intercropping is a typical phytoremediation measure in agricultural management technology (Bian et al., 2021). It is a potential and simple bioremediation method to reduce the accumulation of heavy metals in farmland crops by intercropping crops and heavy metal hyperaccumulation plants (Rathore et al., 2019). However, due to the influence of environmental factors, phytoremediation technology often has problems such as low efficiency and slow speed.

At present, the remediation technology of soil Cr still has some limitations. Most studies only focus on the research of single control technology such as phytoremediation technology and in-situ passivation technology, and do not pay attention to the synergy of integrated technology combining phytoremediation technology and solidification stabilization technology. In this paper, the influence of two carbon source amendments (biochar and modified bagasse), intercropped *Leersia hexandra* and their combinations on pH, Cr content, soil nutrients, enzyme activity and microbial number of Cr contaminated soil were analyzed. The purpose of this study is to explore the effect of the combination of soil amendments and intercropping Cr hyperaccumulators on the passivation of soil Cr, in order to provide reference for the effective use of carbon source amendments and the combined remediation technology of Cr contaminated soil.

Materials and methods

Soil samples and plants collection

The soil used for testing was obtained from the vegetable field at South China Agricultural University, where the top 5-25 cm layer was gathered for analysis. Following the removal of plant residues and stones, the soil was air-dried and subsequently ground to pass through a 2 mm sieve. Subsequently, the prepared soil was placed into individual plastic basins (50 cm in height and 25 cm in diameter), each containing 5 kg of soil. Chromium (Cr) in the form of analytical pure $K_2Cr_2O_7$ was then applied to the soil and thoroughly mixed to achieve a Cr concentration of 50 mg/kg,

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after which the soil was allowed to equilibrate for 2 weeks. Fundamental physical and chemical properties of the soil are presented in *Table 1*.

The water spinach tested was of the green stem bamboo leaf variety grown in Hong Kong, while the *Leersia hexandra* seedlings were collected from the Taohua River in Guilin City, Guangxi Province.

Preparation and characterization of carbon source amendments

The biochar utilized in the study was derived from market peanut shells. These shells were initially crushed and subjected to anoxic pyrolysis at 650°C in a SX-5-12 muffle furnace for 2 h. Subsequently, the resultant black solid was retrieved, ground, and sieved through a 0.147 mm sieve to obtain the biochar. The bagasse used in the study originated from a sugar factory and underwent natural drying followed by sieving through a 50-100 mesh sieve. Following this, 30 g of bagasse powder was combined with 500 mL of 4 g/L NaOH solution in a 500 mL conical flask. The sealed mixture was then agitated for 4 hours at room temperature, with a rotating speed of 60 r/min, until complete reaction occurred. The resulting light yellow filter residue was fully washed with distilled water and subsequently dried at 50°C to yield the modified bagasse required for testing.

Collection of soil samples and plants

The soil sample was obtained from the top 5-25 cm layer of soil in a vegetable field under the purview of the Ecology Department within the College of Natural Resources and Environment at South China Agricultural University. Following the removal of impurities, the soil sample underwent air-drying, grinding, and sieving with a 2 mm sieve. Then, it was allocated into 50 cm black plastic pots with diameters of 15 cm, each containing 5 kg of soil. After adding Cr (in the form of $K_2Cr_2O_7$) at a rate of 50 mg kg^{-1} to each pot, the contents were thoroughly and uniformly mixed. Subsequently, the mixture was soaked in water and left to rest for one month. Following this, soil samples were sequentially collected, weighed, air-dried, ground, and sieved prior to the measurement of their basic physicochemical properties (*Table 1*).

The experiment utilized the narrow-leaved and white-skinned (Hong Kong) *Ipomoea aquatica*, identified as a low chromium (Cr) accumulator. The seeds for this species were purchased directly from a seed market in Guangzhou. In contrast, the seedlings of *Leersia hexandra*, known as a Cr hyperaccumulator, were gathered from the banks of the Taohua River in Guilin, Guangxi Province.

Preparation and characterization of carbon source amendments

The biochar used in this research was derived from peanut straw sourced from the market. The preparation of samples is outlined below. Initially, the peanut straws were cleaned, dried, and then pulverized in a grinder before being placed in a crucible. The crucible, containing the pulverized material, was subjected to a 2-h pyrolysis process at 650°C within an oxygen-limited environment using a muffle furnace (KSY-12D-16). Subsequently, after returning to room temperature, samples were removed, weighed, sieved through a 0.147 mm aperture, and finally stored in a bag.

The bagasse used in this study was derived from sugarcane purchased from a local market. Following the peeling process, the sugarcane juice was extracted using a cane juicer. Subsequently, the bagasse underwent natural air-drying, was pulverized to a

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particle size of 50-100 meshes using a pulverizer, and then stored. For the modification process, 30 g of bagasse powder was placed into a 500 mL conical flask. To this, 500 mL of NaOH solution at a concentration of 4 g L^{-1} was added. The conical flask was sealed with a rubber stopper and allowed to react at room temperature with continuous oscillation (revolving speed: 60 r min^{-1}) for 4 h. After the reaction was complete, filtration was carried out. The resulting light-yellow filter residues were washed with distilled water until reaching neutral pH, followed by drying in an oven at 50°C for 24 h. This process yielded the modified bagasse required for the experiment (Niu et al., 2013). The elemental composition (C, H, N, and S) of the carbon source amendments is detailed in *Table 1*.

Table 1. *Basic physicochemical properties of the carbon source amendments and soil*

Parameters	Biochar	Modified bagasse	Soil
pH	9.75	4.66	6.02
C (%)	65.35	37.3	-
O (%)	17.32	46.76	-
H (%)	2.92	4.91	-
N (%)	1.23	0.29	-
S (%)	0.44	0.11	-
BET Surface area (m^2 g^{-1})	21.56	1.21	-
Mass transfer performance (%)	28.21	4.65	-
Adsorption capacity (mg g^{-1})	27.69	3.34	-
Organic matter (g kg^{-1})	-	-	29.18
Total N (g kg^{-1})	-	-	1.20
Total P (g kg^{-1})	-	-	0.31
Total K (g kg^{-1})	-	-	15.76
Alkali-hydrolyzable N (mg kg^{-1})	-	-	55.94
Available P (mg kg^{-1})	-	-	60.44
Available K (mg kg^{-1})	-	-	135.32
Total Cr (mg kg^{-1})	0.21	-	13.76

Experimental design

The experiment was pot experiment. A total of 8 treatments were set up in the experiment: CK (i.e., no amendment or intercropped *Leersia hexandra*), L (i.e., intercropped *Leersia hexandra*), B (i.e., application of biochar), S (i.e., application of modified bagasse), BS (i.e., simultaneous application of biochar and modified bagasse), SL (i.e., application of modified bagasse in combination with intercropped *Leersia hexandra*), BL (i.e., the combination of biochar and intercropped *Leersia hexandra*), BSL (i.e., simultaneous application of biochar and modified bagasse in combination with intercropped *Leersia hexandra*). The addition of biochar and bagasse was both 20 g kg-1. 4 water spinach plants were planted in each pot under monocropping treatment. 2 *Leersia hexandra* plants and 2 water spinach plants were planted in each pot under intercropping treatment, and each treatment was repeated 4 times, a total of 32 pots. Biochar and modified bagasse were added before plant transplanting and mixed well. *Leersia hexandra* and water spinach were transplanted at the same time. The experiment lasted for 45 days, during which deionized water was added regularly to keep the soil

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water content as 40%~60% of the field water capacity. After the experiment, samples were taken for determination.

Sample collection

After the plants were harvested, soils from the pots were uniformly blended. Soil samples were then taken by means of quartering. Some samples were taken and used to measure the mechanical compositions in soils; as for the remaining samples, they were divided into two portions after grinding and sieving. One portion was stored in a refrigerator at 4°C to measure soil enzyme activity and microbial quantity. In contrast, the other portion was air-dried and used to measure the soil mechanical compositions, organic matter, alkali-hydrolyzable nitrogen, and the content of available phosphorus and potassium.

Physiochemical and biological property determination of soil

A pH meter was used to measure the soil pH (soil solution ratio: 1:2.5); the content of soil organic matter was determined by a potassium dichromate method (Wang et al., 2014), and soil alkali-hydrolyzable nitrogen content was measured by alkaline hydrolysis diffusion (Zeng et al., 2011). In terms of available phosphorus and readily available potassium, molybdenum-antimony resistance colorimetry with $NaHCO_3^-$ (Arif et al., 2018) and flame photometry with NH_4OAc (Yoo et al., 2018) was respectively implemented. Regarding soil bacteria, actinomycetes, fungi, and the content of urease, phosphatase, sucrase, and catalase, they were measured by an approach raised by Borowik et al. (2017).

Structural analysis of amendments

The specific surface area and pore structure of amendment samples were determined by using the specific surface area and aperture tester (ASAP 2460, America Micromeritics company), and the elemental analyzer (vario ELcube, Germany Elementar company) was used to determine the content of N, C and H elements in amendment samples. The functional groups were determined by fourier transform infrared spectrometer (Vertex 70, Bruker). X-ray diffraction (XRD) analysis was carried out with X-ray diffraction (Ulima IV, RIGAKU).

Data processing

The data were managed using Microsoft Excel 2016, and the corresponding charts were made in Origin 8.5. Moreover, a one-way analysis of variance was conducted in SPSS. After performing the test using the Duncan method, the corresponding significance level turned out to be 0.05, i.e., $\alpha = 0.05$.

Results

Characterization of amendments

According to FTIR (*Fig. 1*), there were some differences in the functional groups of biochar and modified bagasse, and the vibration forms of the same functional groups were also different. There was a methyl absorption peak at 2930 cm^{-1} in the modified

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bagasse. There were hydroxyl absorption peak and phenol hydroxyl absorption peak at 3360 cm^{-1} and 1250 cm^{-1}, respectively, in biochar and modified bagasse. However, the peak area of modified bagasse was more extensive than that of biochar, which indicated that the number of hydroxyl and phenol hydroxyl groups in modified bagasse was higher than that of biochar. It proved that the hydroxyl was successfully loaded into bagasse after NaOH modification. There were C = O and aromatic C-H absorption peaks in biochar at 1620 cm^{-1} and 750 cm^{-1}, respectively.

According to the XRD pattern (*Fig. 2*), the wide and slow dispersion peak of biochar appeared in the range of 2θ = 22 ~ 36 °, mainly due to the stacking of a single atom carbon layer in the microcrystalline. The main peak of the modified bagasse appeared at 26.8 ° (2 θ), which corresponded to the diffraction peak of the amorphous structure, mainly related to the crystalline structure of cellulose within biomass (Keiluweit et al., 2010), which indicated that the modified bagasse contains much crystalline cellulose.

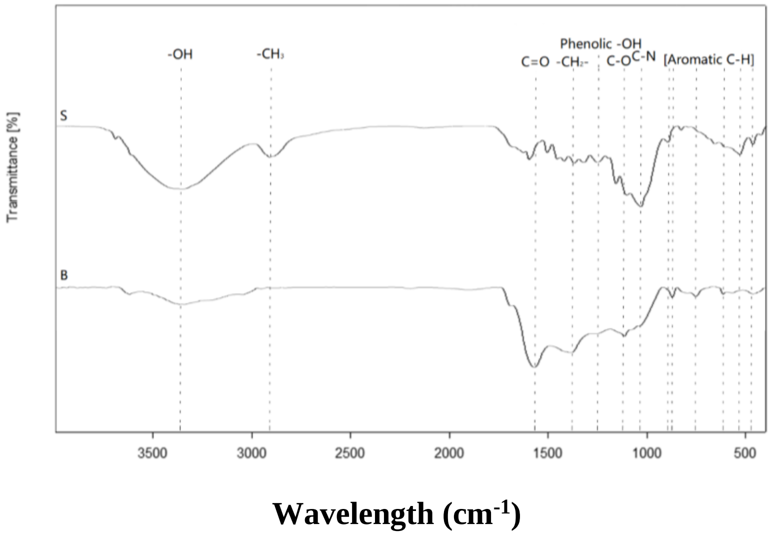

***Figure 1** Fourier transform infrared spectroscopy (FTIR) spectra of bagasse (S) and biochar (B)*

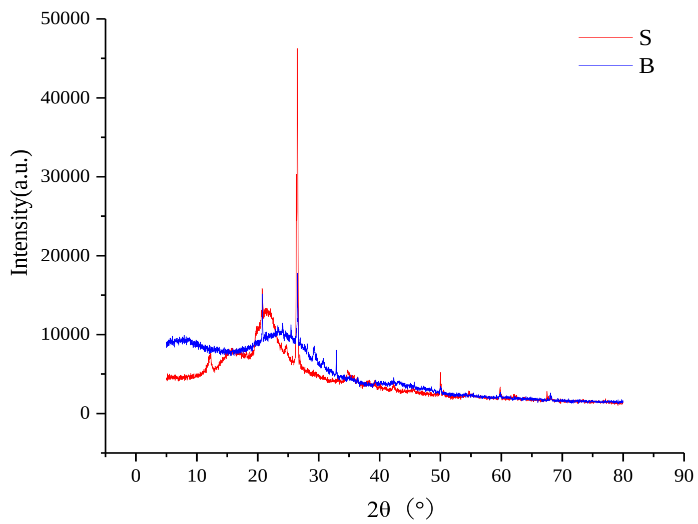

***Figure 2.** The X-ray diffraction (XRD) patterns of bagasse (S) and biochar (B)*

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The soil's mechanical composition

As can be observed from *Figure 3*, two carbon source amendments (biochar and modified bagasse), intercropped *Leersia hexandra*, and their different combinations present different influences on the sand, silt, and clay fractions. In line with the diverse treatment approaches, the soil sand contents were all below those in the control group; its minimum value, which was 12.13% lower than CK, was found in the BL treatment group. Apart from BS and BSL, soil clay fractions generated by other treatment methods were all above those in the CK. Moreover, the maximum clay fraction was achieved from the S treatment, and its maximum value was 12.58% higher than that of CK. In general, the soil mechanical compositions are summarized below as far as the diverse treatment approaches are concerned: sand fraction > clay fraction > silt fraction. In comparison with CK, the fractions of sand, silt and clay all changed significantly in the BL treatment ($p < 0.05$). To be specific, the sand fraction decreased, while the clay fraction was elevated during the treatment with BL.

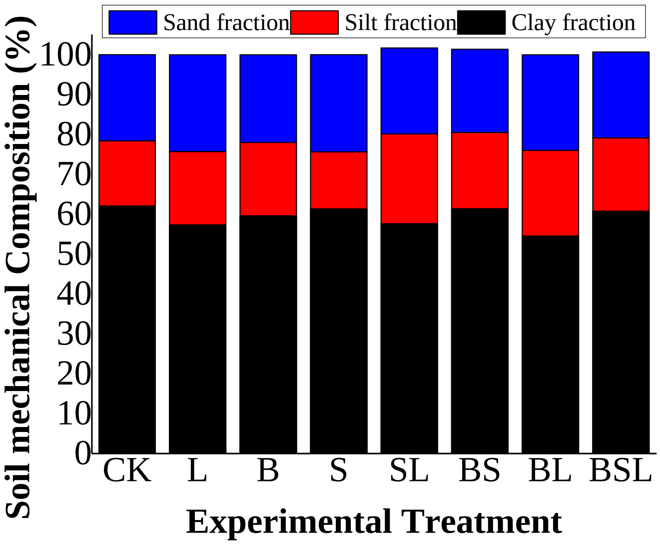

Figure 3. *Sand fractions, silt fractions and clay fractions in soils treated with carbon source amendments, intercropped Leersia hexandra and their different combinations. Letters in this bar chart represent significant differences in the average values generated by different treatment approaches (P < 0.05)*

Physicochemical properties of soils

In contrast with the CK, the contents of organic matters, alkali-hydrolyzable nitrogen, readily available nitrogen and readily available potassium were enhanced to a certain extent in soils treated with two carbon source amendments (i.e., biochar and bagasse residues), intercropped *Leersia hexandra* and their different combinations, as shown in *Figure 4*.

In comparison with CK, soil remediation with only L exerts no significant influence on soil organic matter content ($p > 0.05$); however, soil organic matter content is significantly improved as far as other treatment approaches are concerned, i.e., $p < 0.05$. Among them, soil treatment with BL produced the most satisfactory effects on the content of soil organic matter. To be specific, their content increased by 54.34% compared with that generated by CK. In the same aspect, soil organic matter content

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increase caused by soil remediation with B came second; compared with the CK, it rose by 33.66% compared with that generated by CK (*Fig. 4A*). Soil treatments with L, SL, and BL all significantly enhanced the content of soil alkali-hydrolyzable nitrogen ($p < 0.05$). Regarding other treatment approaches, no significant difference was found when compared with CK, i.e., $P > 0.05$. The differences between BL and L treatments were not statistically significant based on *Figure 4B*. Compared with CK, three soil remediation approaches that involve B (i.e., B, BS, and BL) all significantly improved the content of readily available phosphorus and potassium in the soils ($p < 0.05$); however, the insignificant influence was exerted by other treatment approaches on their content ($P > 0.05$). To be specific, the highest increase in readily available phosphorus content was generated by BS. Regarding the rise of readily available potassium content, BL performed best, followed closely by B. Respectively, the corresponding increments for readily available potassium content were 59.29% and 57.99% (*Fig. 4C, D*).

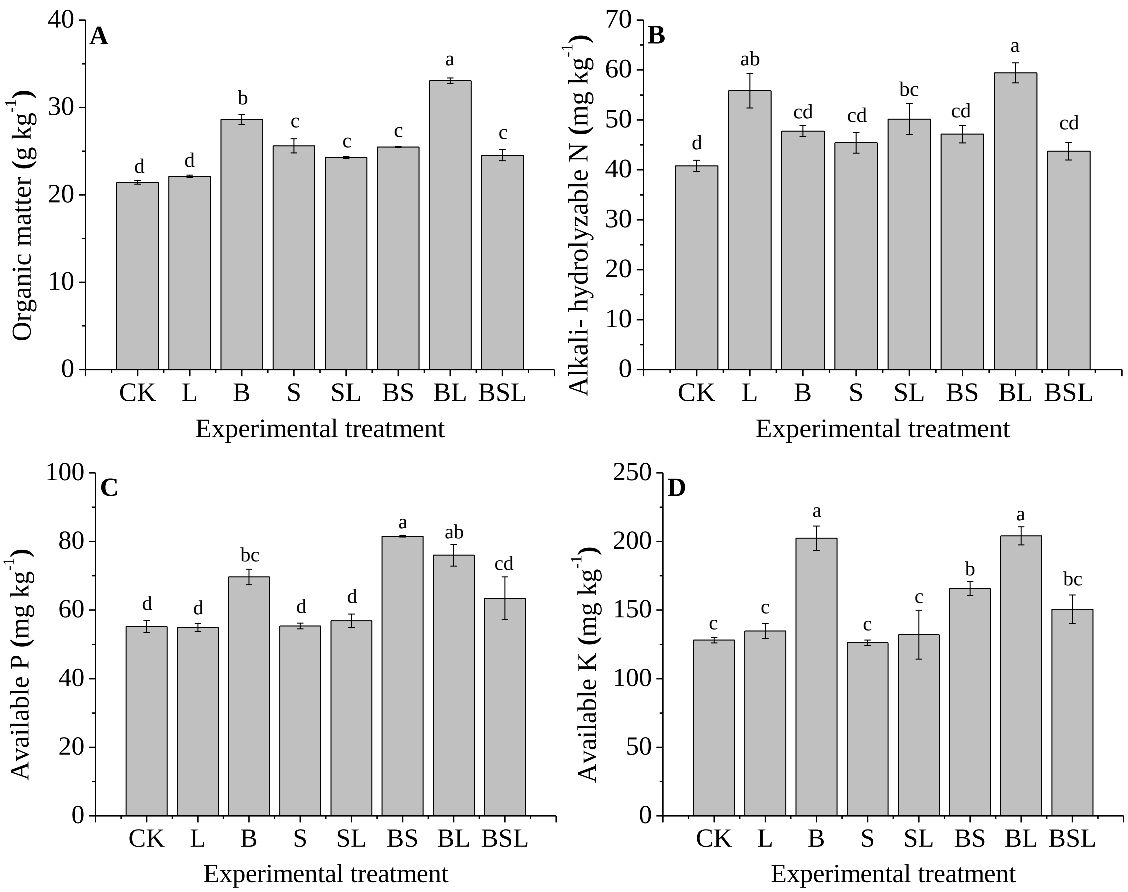

Figure 4. *Influence of soil remediation with carbon source amendments, intercropped Leersia hexandra and their different combinations on the content of soil organic matter (A), alkali-hydrolyzable nitrogen (B), readily available nitrogen (C) and readily available potassium (D). Letters in this bar chart represent significant differences in the average values generated by different treatment approaches (P < 0.05)*

According to these findings, the best soil remediation effects were produced by BL, which is embodied in the significant increases of organic matter, alkali-hydrolyzable nitrogen content, and readily available potassium in soils. As for the increment in

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readily available phosphorus content in soils, the maximum rate was generated by BS. This signifies that biochar application, *Leersia hexandra* intercropping, and biochar-related combinations show a certain degree of synergism.

Soil enzyme activity

Under the circumstance that the soils were treated with two carbon source amendments, intercropped *Leersia hexandra* and their different combinations, urease activities in such soils were elevated by 22.82-50.54% in comparison with CK. Compared with CK, BL showed the capability to significantly enhance soil urease activity ($p < 0.05$), but no significant effect was achieved by other treatment approaches ($P > 0.05$) (*Fig. 5A*). Compared with the control group, phosphatase activity was improved by 6.79-32.66% under different treatments. In comparison with CK, B and BL can significantly elevate phosphatase activity in soils ($p < 0.05$); however, no significant differences in phosphatase activity were found between either B or BL and CK ($P > 0.05$) (*Fig. 5B*). Compared with the control group, catalase activity in soils rose by 10.00-37.69% for all treatment approaches. Compared with CK, while soil treatment with L and BL significantly improved catalase activity ($p < 0.05$), no significant influence was exerted by other approaches ($P > 0.05$) (*Fig. 5C*).

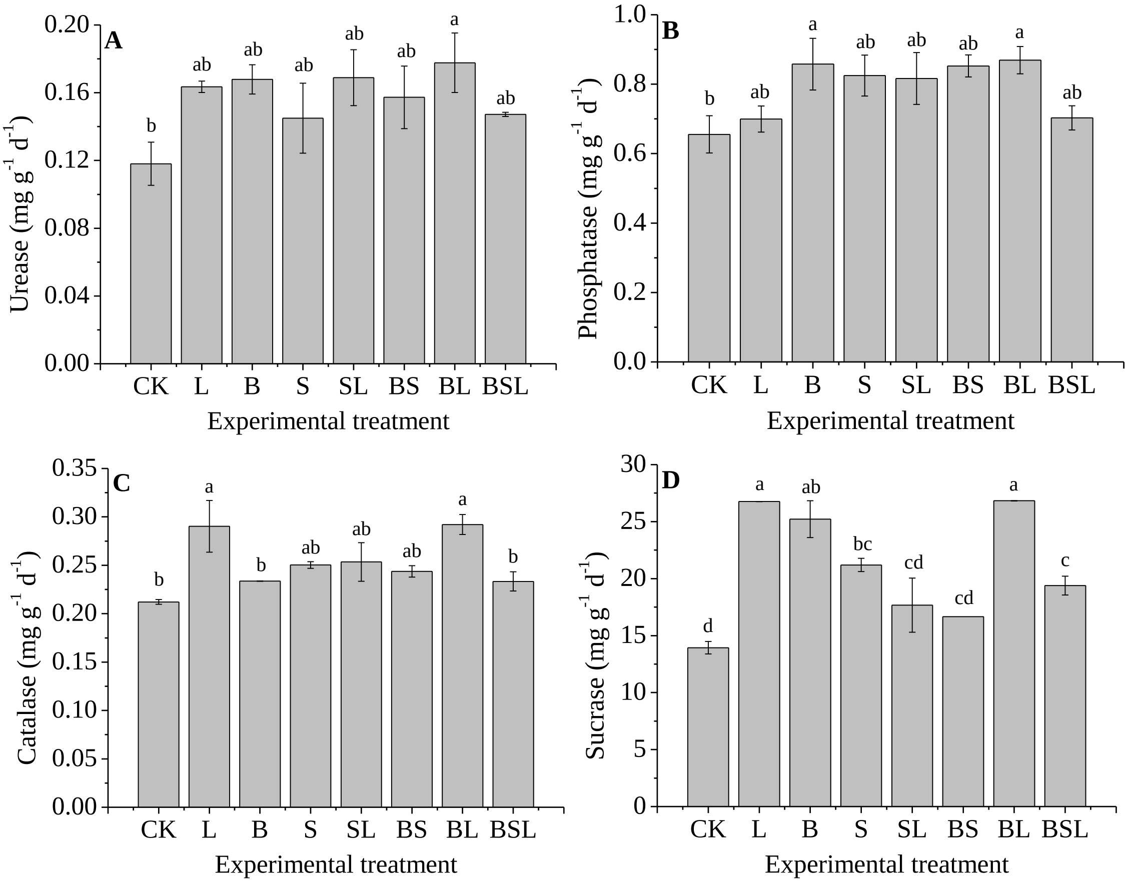

Figure 5. *Influence of soil remediation with carbon source amendments, intercropped Leersia hexandra and their different combinations on the activity of urease (A), phosphatase (B), catalase (C) and sucrase (D) in soils. Letters in this bar chart represent significant differences in the average values generated by different treatment approaches (P < 0.05)*

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In comparison with CK, sucrase activity in soils treated with all approaches increased by 19.51-92.55%. The influences of SL, BS, and BSL on sucrase activity were insignificant ($p > 0.05$), and soil remediation with L, B, S and BL all significantly enhanced the sucrase activity ($p < 0.05$). Among them, soil treatment with BL produced the highest sucrase activity increase, followed by the treatment with L. Compared with the control group, treatments with BL and L increased by 92.55% and 92.05% respectively (*Fig. 5D*). Accordingly, BL outperformed other treatment approaches in improving the activity of urease, phosphatase, catalase, and sucrase in soils.

Microbial quantity of soils

In comparison with CK, all treatment approaches can increase the number of bacteria, actinomycetes, and fungi in soils by 13.41-79.96%, 3.65-51.22%, and 8.45-109.77% respectively, as shown in *Figure 6*.

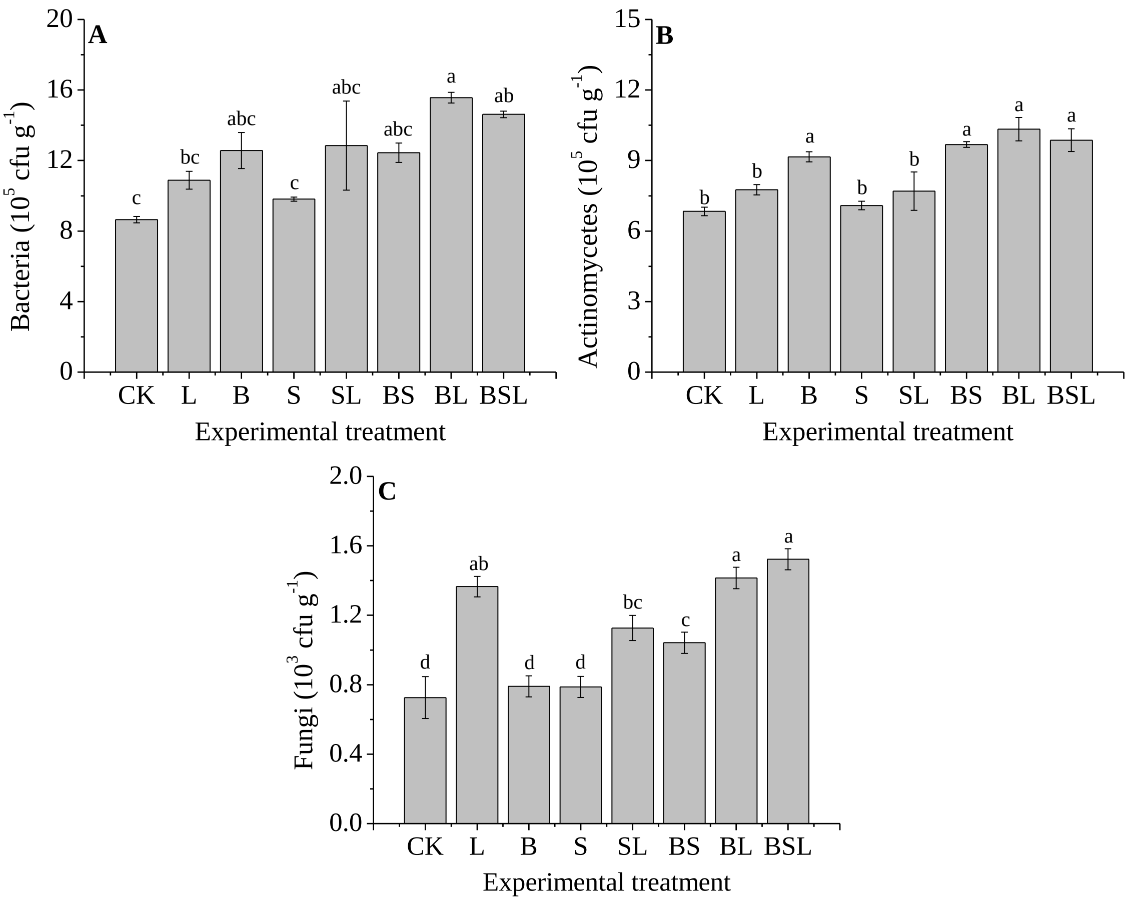

Figure 6. *Influence of soil remediation with carbon source amendments, intercropped Leersia hexandra and their different combinations on the number of soil bacteria (A), actinomycetes (B) or fungi (C). Letters in this bar chart represent significant differences in the average values generated by different treatment approaches (P < 0.05)*

The highest increase of soil bacteria quantity was found in soil remediation with BL results with BSL took second place. Subjected to such two treatments, the quantities of

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soil bacteria were significantly different from CK ($P < 0.05$), has increased by 79.96% and 68.99% respectively. As for the influence of other treatment approaches on the number of soil bacteria, they appeared to be insignificant ($P > 0.05$), as shown in *Figure 6A*. Under various treatments, the quantity of soil actinomycetes increased by 3.65-51.22% compared with that of CK. Moreover, a significant rise of such a quantity could be found from soil treatments with B, BS, BL, and BSL ($P < 0.05$). The most significant increase in the quantity of soil actinomycetes was generated by BL, followed by the soil treatment with BSL. Respectively, they increased by 51.22% and 44.35% (*Fig. 6B*) compared with that of CK. All soil treatment approaches enabled the number of soil fungi to increase by 8.45-109.77%. Apart from the fact that the treatment with B or S applies an insignificant influence on the soil fungus quantity ($p > 0.05$), other treatment approaches demonstrated a significant ability to increase the soil fungus activity ($p < 0.05$). For instance, the most considerable increase of fungus quantity was generated by treatment with BSL; compared with CK, its increase rate was up to 109.77%. Then, the increase rate (i.e., 94.95%) produced by treatment with BL took the second place, as shown in *Figure 6C*. Accordingly, BL gave rise to the most significant increase in the number of soil bacteria and actinomycetes in soils; and, the most substantial increase in the soil fungus quantity was generated by soil remediation with BSL.

Discussion

As an important indicator of soil classification, mechanical composition affects not only material migration and transformation but also migration and bioavailability of heavy metals (Fijałkowski et al., 2012). This study shows that the proportion of clay in the soil is significantly increased by a combination of biochar and intercropping. A higher clay fraction may result in a higher number of large aggregates according to relevant research (Kristiansen et al., 2006; Wick et al., 2009). Biochar also promotes soil aggregation formation (Ouyang et al., 2013) and can be described as follows in the corresponding possible mechanism. While the surface of biochar is attached to minerals, organic matter, and root exudates, intercropping encourages root exudates to be produced in the soil and has different molecule dimensions and chemical characteristics. It is supposed to minimize the surface area of biochar on a molecular level and thus become a binder for the formation and the stabilization of soil aggregates (Liang et al., 2006). Additionally, this study further shows that the two carbon source amendments all increased the content of organic matter, alkali-hydrolyzable nitrogen, readily available nitrogen, and readily available potassium in Cr-contaminated soil. The content of soil alkali-hydrolyzable nitrogen is significantly increased with intercropped *Leersia hexandra* alone, but no significant influence on organic matter content, readily-available nitrogen or potassium. A combination of intercropping and biochar has contributed to the highest increase in the content of these nutrients, as the eluvial loss of nutrients on the soil is easier for Biochar. Possible explanations for such a phenomenon are that biochar possesses adsorption properties on the one hand, while biochar applied to the soil, due to abundant organic macromolecules and the formation of pore structures can easily facilitate the production of large aggregates. Thus, biochar is specifically allowing adsorption and retention of nutrient ion in soils to be enhanced (Zheng et al., 2010). This equates to the fact that biochar and intercropping combinations can stimulate the development of soil aggregates.

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Soil enzymes are deemed as critical soil constitutions closely related to the physicochemical properties of soil (Siebielec et al., 2018). The activity of soil enzymes is severely affected by environmental pollution (Karaca et al., 2010), particularly soil urease, soil phosphatase and soil catalase. In this study, the activity of soil urease, phosphatase, catalase and sucrase in both biochar and intercropped *Leersia hexandra* is considerably increased, and their combined impact has the most significant effect on soil enzyme activity. Our results coincide with Yang et al. (2013), who reported that the activity of soil urease, neutral phosphatase and sucrase after biochar treatment increased by 31.1-37.6%, 29.7-193.8%, and 36.5-328.6%, respectively, compared with the control group. Biochar can adsorb a variety of organic and inorganic molecules in soil and affect the enzymic activity by adsorbing such molecules and/or their substrates (Jin, 2010). There have been research findings that enzyme adsorption properties of biochar depend on several factors, including additional levels of biochar, the content of soil nutrients, and specific enzymes (Wang et al., 2015). Comparatively, some other investigations have also pointed out that biochar plays a certain role in suppressing the activity of some soil enzymes (Tang et al., 2020). Their argument seems to be contradictory, likely because of a difference between raw materials (Tang et al., 2020) and application dosage of biochar (Liang et al., 2020). Therefore, the enzyme adsorption action of biochar should be more profoundly explored. In addition, the rise of soil enzyme activity is relevant to microbial quantity in soils and the increase in the activity of soil microbes (Zhang et al., 2023).

Biochar and intercropping combination are best performed in the current study to increase the number of soil bacteria and actinomycetes. In relation to the potential mechanism of the influence of biochar on soil microbes, two aspects can be developed. In order to protect and encourage their growth, one is to provide a carbon substrate (Smith et al., 2010), and in addition to providing habitat to microbes (Quilliam et al., 2013). With regard to intercropping, the activity of soil enzymes and the improvement in the number of soil microbes is often reported (Zhou et al., 2011; Li et al., 2013). Furthermore, both the enzymatic activity and the microbial quantity are improved by the proposed modified bagasse as a kind of carbon source modification. In specific terms, the most significant impact of the combination of Biochar + Bagasse + Intercropsed *Leersia hexandra* is to increase the number of soil fungi. A primary explanation may be that bagasse acts as a composting material and introduces more external microorganisms, enzymes, and available nutrients into the soil. This means that bagasse can provide a suitable habitat for microorganisms (Liang et al., 2020). Hence, such alterations result in improvement of activity and biomass of microorganisms and enzymes, in an adjustment of microbial community structures, and the changes of composing proportions of microorganisms such as bacteria and fungi (Abbott et al., 2018; Liang et al., 2020).

Conclusions

In Cr polluted soils, carbon source amendments (biochar and modified bagasse), intercropped *Leersia hexandra* and their different combinations all changed the soil mechanical composition, improved the physicochemical and biological properties of the soil. This combination of technology could improve the soil characteristics. It can be used as an effective measure to the improvement of the safe crop production and the

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remediation of Cr polluted soil, which is in line with the efficient and sustainable remediation concept of considering both production and remediation.

It is worth mentioning that in the process of the practical application of this technology, we should pay attention to adjust the combination of amendment and hyperaccumulator varieties according to the crop type and soil pollution, and further study is on the way.

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