

Effects of Biochar on Resistance Genes During Remediation of Arsenic and Cadmium Contaminated Soils

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Abstract

In order to explore the impact mechanism of remediation materials on ARGs and MRGs in soil under the condition of heavy metal As and Cd pollution, and put forward the application strategy of biochar to control ARGs, this project plans to use biochar to repair As and Cd contaminated soil, and design three treatments, CK (blank non polluted soil) H (As (100mg/kg)+Cd (3mg/kg)), HB (As (100mg/kg)+Cd (3mg/kg)+biochar (5%)), to analyze the physical and chemical properties of soil, The microbial diversity in soil was detected. The abundance of Streptomycetes, Propionibacteriales and Bacillales in HB treatment increased by 7.0%, 4.2% and 13.3% compared with D0. The characteristics of biochar weaken most of the cycling functions of soil microorganisms. RpoB, MtrA, VanRO, ARO: 3004480, parY are the five gene types with the highest abundance in the experimental samples, of which VanRO is a vancomycin resistance gene, and the addition of biochar hinders the reduction of this type of antibiotic resistance gene.

Keywords

Biocha; Resistance Genes; Arsenic; Cadmium; Contaminated Soil.

1. INTRODUCTION

As an important part of soil ecology, soil microorganisms control the decomposition and transformation of various nutrients in soil. Soil microorganisms are closely related to the soil environment. Soil microorganisms are more sensitive to changes in the soil environment. When the soil ecological environment changes, soil microorganisms will react quickly, which is reflected in changes in soil microbial abundance and community structure. Therefore, soil microbial richness, diversity and microbial community structure are one of the important indicators for evaluating soil fertility and soil environment.

At present, there are different views on the impact of biochar on soil microorganisms. Some studies believe that applying biochar to soil can increase soil fertility, and its porous structure can provide protection for microorganisms, so biochar will have a more positive effect on soil microorganisms. Some researchers also believe that biochar will produce many harmful substances in the preparation process, which has a relatively adverse impact on soil microorganisms. At the same time, the effects of biochar on soil bacteria and fungi are also significantly different. Wang Caiyun et al. found that the application of biochar significantly increased the number of soil bacteria and bacteria/fungi ratio, reduced the number of fungi and *Fusarium oxysporum*, and changed the soil type from fungal to bacterial, especially for the soil with 10-year continuous cropping. The ratio of soil bacteria and bacteria/fungi was 2.00 and 3.64 times of that of untreated soil, respectively, and the number of fungi and *Fusarium*

oxysporum was 54.8% and 55.9% of that of untreated soil. Different raw materials of biochar will have different effects on soil after being applied to soil, and the proportion of biochar applied to soil will also have different effects on microorganisms. Chen Yixuan et al. found that the application of peanut shell biochar, corn stalk biochar and poplar sawdust biochar can promote the total soil microbial biomass and soil bacterial biomass, but the increase rate decreases with the increase of biochar concentration, while the addition of bamboo sawdust biochar can significantly reduce the total soil microbial biomass and soil bacterial biomass. Cheng Yang et al. found that the addition of biochar with different content had a great difference in the change of soil microbial community structure and function. Compared with the application of 10t hm⁻² treatment, the application of 5t hm⁻² treatment had a more significant effect on soil microbial community.

Macrogenomes are characterized by high flux, fast speed and complete information. They have great advantages in identifying low abundance microbial communities and mining more gene resources, such as functional genes and functional proteins. At present, as one of the important ways to study the relationship between microorganisms and the environment, metagenomics has attracted the attention of scholars in all aspects. Tian explored the process of heating anaerobic digestion of sewage sludge through metagenome technology. After high-temperature treatment, the contents of resistance genes, resistance gene hosts and mobile elements of sludge were significantly reduced. Hazen used the macro genome technology to screen the effect of oil pollution on environmental microorganisms and the functional genes of microbial remediation.

This project mainly aims at the problem of microbial community structure and ARGs residue in As and Cd contaminated soil. With biochar as the remediation material, the method of combining experiment and theory is adopted to explore the impact of remediation materials on soil ARGs; Analyze the relationship between soil microbial community and ARGs change, reveal the main control factors and microbial mechanism of remediation materials affecting ARGs, and propose remediation materials suitable for ARGs abundance and improving soil quality.

2. MATERIALS AND METHODS

To study the effects of remediation materials on microbial communities and resistance genes in heavy metal contaminated soil, Biochar is proposed to be used in this project to study the remediation of As and Cd contaminated soil. The specific test design. The treatment is as follows: CK: blank uncontaminated soil, H: blank soil+As (100mg/kg)+Cd (3mg/kg), HB: blank soil+As (100mg/kg)+Cd (3mg/kg)+biochar (5%).

Plants shall be planted in plastic pots. 1.5kg soil (0-20cm farmland soil) shall be put into each pot, and the soil surface shall be 2cm away from the pot mouth after loading. The ryegrass seeds were placed in a wet culture dish, and germinated in a constant temperature incubator (37 °C) for 48-72h. The seedlings of the test plants with consistent growth were selected and transplanted into the control and each treatment pot, with 6 plants in each pot and 3 repeats in each treatment. During the pot experiment, the soil moisture was maintained at 60% of the field capacity. The daytime temperature in the glass greenhouse was 30 °C, and the nighttime temperature was 22 °C. Exchange the position of the pot in the greenhouse every 2 days to ensure the same culture conditions for potted plants. Pot experiment was conducted in glass greenhouse for 60 days, and all plants and soil were harvested after 60 days of experiment.

3. RESULTS AND DISCUSSION

3.1. Impact Analysis on Soil Physical and Chemical Properties

Table 1. Physical and Chemical Properties of Soil Treated

Treatment	Organic matter / (g/kg)	TC / (g/kg)	TP / (g/kg)	AP / (mg/kg)	AK / (mg/kg)
D0	4.98	0.63	0.68	30.9	288.8
CK	11.26	0.58	0.38	61.4	278.8
H	14.54	0.64	0.52	78.2	288.1
HB	22.36	1.14	0.85	117.4	674.5

As shown in Table 1, the results showed that the application of biochar humic acid significantly increased the content of organic matter, total nitrogen, total phosphorus, and total potassium in the chromium contaminated soil, which may be related to the biochar's ability to reduce the leaching of some nutrients in the soil, on the one hand, due to the adsorption characteristics of biochar itself; On the other hand, it may be that biochar contains rich organic macromolecules and developed pore structure, which is easy to promote the formation of large aggregates after being applied to the soil, thus enhancing the adsorption and retention of soil nutrient ions. Biochar contains a large number of stable carbon elements, which can be stored in the soil for a long time and is not easy to decompose. The total nitrogen in the soil presents HB>H>D0>CK. Zhang Hanzhi and other researchers found that the application of biochar can significantly increase the organic matter content and total nitrogen content of red soil, and the total phosphorus and potassium content in red soil have no significant changes compared with the control, which is not consistent with the results of this experiment. In this experiment, the total phosphorus and total potassium of soil under each treatment were significantly increased compared with the control, which may be due to the interaction of various nutrient elements in biochar and inorganic fertilizer, which activated the nutrient activity in the soil and improved the surrounding environment for crop growth and development.

3.2. Diversity Analysis of Soil Microbial System

In this study, Illumina Novaseq platform conducted double ended sequencing on sequencing samples, and the number of effective sequences of different treatments reached more than 95%. These samples broadly represented the diversity of microbial community structure, which was used to analyze the impact of biochar on the microbial community structure of As and Cd contaminated soil. Table 2 lists the sequencing sequence information of samples from different treatments. The number of effective sequences varies among different treatments, in which As and Cd pollute the soil.

Table 2. Sequence information of samples from different treatments

Treatments	Reads	Raw Base(GB)	Clean Reads	Cleaned(%)
D0	27814709	8.34	26752062	96.18
CK	29168588	8.75	27868441	95.54
H	25101005	7.53	23916747	95.28
HB	28579562	8.57	27322355	95.60

This shows that the total number of OTUs in all six samples is 2536, 209 are unique to D0, 46 are unique to CK, 23 are unique to H, 47 are unique to HB, 47 are unique to HBN, and 41 are

unique to HN. This result shows that the diversity of microorganisms in the initial soil is relatively high. After the planting of ryegrass, the diversity of microbial community structure in the soil decreases, and the presence of As and Cd will further reduce the microbial diversity. After the restoration of biochar and nano zero valent iron, the unique OTU number in HB treatment is basically the same as that in CK treatment, which indicates that biochar and nano zero valent iron can significantly affect the diversity of soil microbial community and have a significant impact on As. The presence of Cd has a repairing effect.

3.3. Overview of Resistance Gene Abundance

Based on the table of relative abundance of resistance genes, calculate the content and percentage of ARO in each sample, and screen out the top 20 ARO results of maximum abundance, as shown in the figure below.

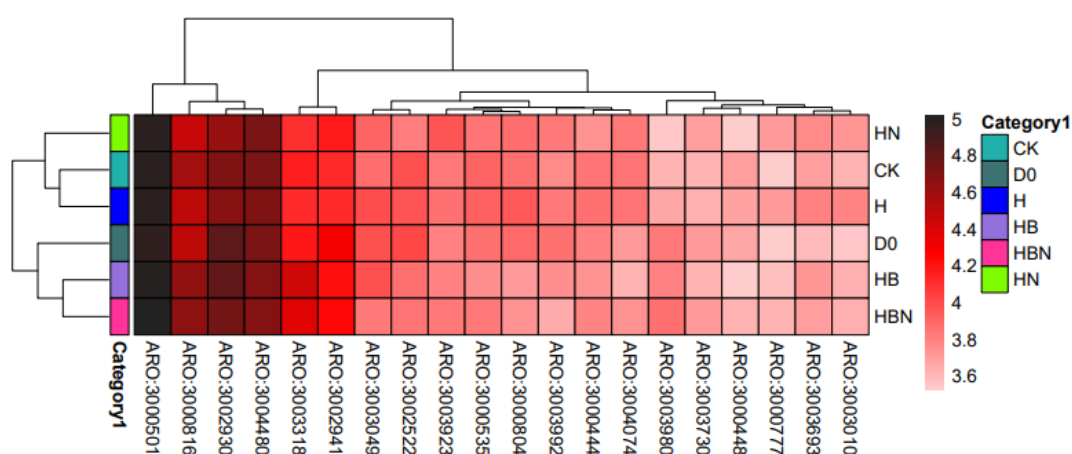


Figure 1. Heat Map of Resistance Gene Abundance

RpoB, MtrA, VanRO, ARO: 3004480, parY are the five gene types with the highest abundance among the experimental samples, and there are large differences in abundance among different treatments. MtrA gene plays a key role in electron transfer. VanRO is a vancomycin resistant gene, which has a high abundance in D0 treatment. After pot planting, the abundance has decreased. However, the addition of biochar has hindered the reduction of this type of antibiotic resistant gene. The reason may be that the biochar increases the abundance of host microorganisms for this type of resistant gene.

4. CONCLUSION

The addition of 5% biochar has better repair effect. The diversity of microorganisms in the initial soil is relatively high. After the planting of ryegrass, the diversity of microbial community structure in the soil decreases, and the presence of As and Cd will further reduce the microbial diversity. After the restoration of biochar, the unique OTU number in HB treatment is basically consistent with that in CK treatment, which indicates that biochar and nano zero valent iron can significantly affect the diversity of soil microbial community and repair the existence of As and Cd.

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