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Impacts and mechanisms of biochar on soil microorganisms

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Abstract: Biochar is a carbonaceous material derived from the pyrolysis of carbon-rich biomass that has attracted increasing research and attention because of its ability to enhance soil carbon storage, increase soil fertility, fix and transform pollutants in soil, and improve the soil environment. These enhancements directly or indirectly affect soil microorganisms' metabolic activities and community structure. This paper reviews the effects of biochar on soil physicochemical properties, enzyme activities, nutrients, contaminants, and related microbial activities. In addition, this work summarises the possible mechanisms involved in the interaction between biochar and microorganisms and the potential hazards associated with biochar use. Finally, this study aims to provide a theoretical basis for future related research.

Keywords: charcoal; soil pollution; soil microbial community; soil bacteria

Biochar is an environmentally friendly material with excellent adsorption properties and high stability that is widely used in the field of water and soil pollution control (Zhao et al. 2020, Xu et al. 2021). In recent years, biochar has attracted considerable attention because of its ability to improve the soil environment and its positive impact on the soil microbial community (Zhou et al. 2020). Soil is a highly complex habitat that contains many soil microorganisms. It is important to investigate biochar's effects on these microorganisms' activities and the subsequent impacts on the soil environment and the sustainable development of agricultural production (Qin et al. 2021, Zhu et al. 2021). Biochar promotes the growth of microorganisms, thus increasing their numbers and abundance (Gou et al. 2018). Moreover, the nutrients contained in biochar, such as carbon (C) and nitrogen (N), are important for increasing the diversity and abundance of most soil microbial communities (Rao et al. 2016). The increased soil nutrient content following biochar application is also a major factor in enhancing microbial metabolic activities (Rao et al. 2016). The water-holding capacity and the pore structure of biochar provide a good habitat for soil microorganisms and are conducive to their growth and reproduction (Chen et al. 2021). In addition, the high adsorption capacity of biochar, which is due to its high specific surface area, can immobilise pollutants in the soil, reduce the migration and transformation rates of pollutants in the soil, and reduce the toxic effect of pollutants on microorganisms (Sun et al. 2021a,b). The raw materials used in the preparation of biochar and the application dose have significant effects on soil microorganisms (Zhou et al. 2020). Kuang et al. (2012) found that biochar effectively increased the biomass of microorganisms in the

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soil, and the biomass of microorganisms in the experimental group increased with increasing doses of biochar compared to the control. The pH and pyrolysis temperature of biochar also had significant effects on the soil microbial community. Prayogo et al. (2014) found that the application of biochar increased the soil pH, resulting in a significant increase in the abundance of the bacterial community. Biochar prepared under low-temperature (less than 500 °C) pyrolysis conditions contained more organic matter and contributed to the formation of soil aggregates, which improved the soil environment and had a significant effect on the diversity and abundance of the soil microbial community (Dai et al. 2017, Ippolito et al. 2020, Tomczyk et al. 2020). Biochar properties vary greatly among biochar prepared under different conditions, and the effects on the soil environment and microorganisms also differ. Differences in the response mechanisms of microorganisms to biochar have been observed. These differences are mainly reflected in the abundance of microbial communities, their community structures, and their metabolic changes (Zhu et al. 2017), and they provide more research directions and ideas for the application of biochar in the soil environment.

This paper reviews the latest research, both in China and internationally, summarises the effects and mechanisms of biochar on soil microorganisms, analyses the possible potential hazards of biochar, and discusses the application of biochar for soil physicochemical properties improvement. The results of this review provide a reference for future research related to biochar application in the soil environment.

Biochar provides a habitat for soil microorganisms

Biochar is a pyrogenous organic material synthesised through the pyrolysis of different types of biomass. Biochar has a variety of effects on the soil environment (Figure 1). The porous structure of biochar is an important feature that can provide a habitat for microorganisms and can protect soil microorganisms from predation (Wang and Zhou 2013). The pore size of biochar (> 50 nm) is usually larger than most soil bacteria, fungi, and protozoa, allowing biochar to accommodate these soil microorganisms, but its pore size excludes large arthropods, which provides conditions for microorganisms to inhabit and colonise without the threat of predation (Wong and Ogbonagya 2021). Pietikäine et al. (2000) found that the colonisation of soil bacteria on biochar reduced the negative impacts of soil nutrient loss due to leaching and increased soil bacteria abundance, but also that biochar had no significant impact on the abundance of soil fungi, which may have been due to the weakened mobility of fungal mycelia caused by its reticular structure. Warnock et al. (2007) found that the pore structure of biochar provided some protection to endomycorrhizal (AM) and ectomycorrhizal (EM) fungi from external environmental stress, and biochar also stimulated the germination of AM and

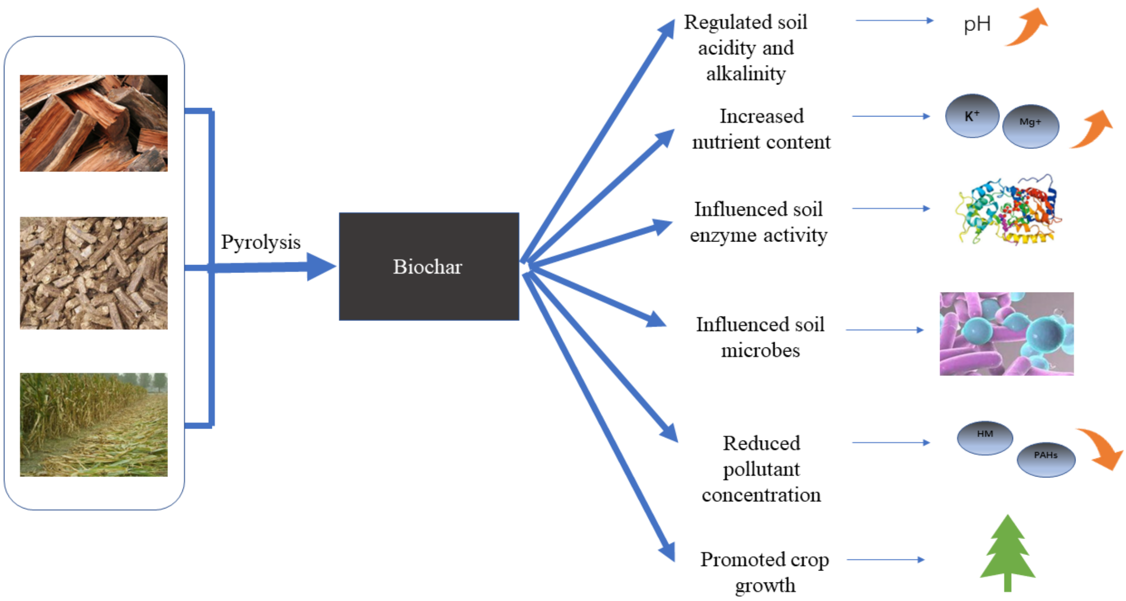

Figure 1. Environmental effect of biochar on soil

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EM fungal spores, which facilitated the growth and reproduction of AM and EM fungi.

The rate of microbial colonisation of biochar is related to the degree of aging of the biochar. Freshly applied biochar may release some endogenous contaminants, such as Cd and Pb, into the soil, which can weaken the metabolic activity of bacteria and slow their colonisation rate on the biochar (Xiang et al. 2021). Unlike fresh biochar, aged biochar helps bacteria and fungi to grow and multiply because it releases fewer contaminants, and the environment created makes it easier for bacteria and fungi to colonise the biochar (Mukherjee et al. 2014, Wang et al. 2017). The co-interaction between microorganisms also affects the time and rate of colonisation; for example, bacterial cells co-cultured with Geobacter metallireducens and Methanosarcina barkeri colonise the biochar surface within a very short time (Zhu et al. 2017).

Mechanisms behind the effects of biochar on soil physicochemical properties and microbial communities

Biochar has alkaline qualities and can raise the pH of the soil, especially in acidic soils. Palansooriya et al. (2019) found that biochar increased soil pH and had a positive impact on the metabolic activity of soil microorganisms and their community structure. The type of raw material used for biochar production is a major factor in determining its pH, and biochar made from wood usually has a higher pH than that made from other materials and is more effective in regulating soil pH. The temperature of pyrolysis also affects the pH of biochar, with biochar produced under high-temperature pyrolysis conditions tending to have a higher pH than biochar produced under low-temperature conditions (Al-Wabel et al. 2013, Gul et al. 2015, Wang et al. 2020). Biochar has a significant effect on microorganisms in acidic soils. Sheng and Zhu (2018) found that the application of biochar in acidic soils significantly increased the pH of the soil and changed the composition of the soil microbial community dramatically, with significant increases in Bacteroides and Gemmatimonadetes and a significant decrease in Acidobacteria and that the regulation of soil pH by biochar was the main reason for this change in the soil microbial community structure (Gorovtsov et al. 2020).

The density of the soil and the stability of agglomerates are affected by the application of biochar, thus directly or indirectly affecting soil microorganisms (Lehmann et al. 2011, Blanco et al. 2017, Chen et al. 2018). Biochar reduces soil bulk density, increases the water-holding and moisture-holding capacity of the soil, affects nitrogen dioxide (N_2O) emissions from the soil, and has an impact on soil nitrifying and denitrifying bacterial communities (Liu et al. 2017a,b). Biochar application also inhibits methane production in the soil (Jia et al. 2012). Initially, biochar intervention does not directly inhibit methanogenic bacteria, but changes in the aeration properties of the soil under biochar application lead to a decrease in the activity and abundance of methanogenic bacteria and an increase in the activity and abundance of methanophilic bacteria, thereby directly affecting CH_4 emissions (Chen et al. 2018, Wang et al. 2019). The application of biochar can affect the alteration of soil agglomerate structures. Zheng et al. (2018) found that when fungi and actinomycetes colonised the surface of biochar particles, their mycelia contributed to the aggregation of soil particles toward the biochar, increasing soil aggregation and making it easier for soil particles to form large-particle agglomerates. This process has a protective effect on the growth of fungi and actinomycetes (Lwin et al. 2018, Gorovtsov et al. 2020).

The application of biochar alters the nutrient content of the soil, which in turn influences microorganisms. The cation exchange capacity (CEC) is an important indicator of soil nutrient retention and fertility and is closely related to the abundance of soil microorganisms and plant growth. Biochar is more effective in improving the CEC in soils with low organic matter content (Lehmann 2007, Zhu et al. 2017). The enhancement of CEC under biochar application can increase the abundance of nitrogen-fixing bacteria, rhizobacteria, and nitrifying and denitrifying bacteria in the soil, promote plant growth and improve the ability of plants to cope with environmental stresses (Glaser et al. 2002, Qin et al. 2021). Biochar also affects the content of other nutrients in the soil as well as the microbial community. Gao et al. (2021) showed that biochar application increased the potassium content in the soil and affected the community structure of fungi because biochar adsorbed nutrients and provided space for fungi to grow, thus increasing their relative abundance. Zheng et al. (2016) found that biochar application increased the total nitrogen content in paddy soils and changed the community structure of soil microorganisms, with bacterial communities being less affected while

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Ascomycota and Basidiomycota communities were more affected. Ammonium nitrogen and nitrate nitrogen had different effects on the abundance of Verrucomicrobia, with soil Verrucomicrobia being negatively correlated with soil ammonium nitrogen and positively correlated with soil nitrate nitrogen (Hu et al. 2019). Gao et al. (2017) found that biochar addition increased the available phosphorus content in the soil, increased the number of soil microorganisms, and influenced the structure of bacterial and fungal communities in tobacco plantation soil, in which the proportions of Actinobacteria and Ascomycota decreased, while the proportions of Proteobacteria, Acidobacteria, Zygomycota, and Basidiomycota increased. Lei et al. (2016) found that the application of biochar increased the total soil carbon content and altered the number of microorganisms in the soil, with a significant increase in fungal abundance. This confirmed that the alteration of soil carbon sources by biochar affected the metabolic activities of soil microorganisms. In conclusion, biochar has a wide range of applications in the augmentation of soil nutrients and the soil microenvironment, and the study of the effects of biochar on soil nutrients and microorganisms can provide a theoretical basis for the study of soil microbiology.

Effects of biochar on soil enzyme activity and microorganisms

The response of soil enzymes to biochar is highly variable, and their activity is influenced by the amount of biochar applied, the type of biochar, and the nature of the soil to which it is applied (Xu et al. 2019). Researchers found that the activity of urease and sucrase in the soil increased significantly with increasing doses and concentrations of biochar because biochar application increased the organic matter and nutrient contents in the soil, which created the conditions necessary for the growth of soil microorganisms and increased the activity of soil microorganisms, as well as the activity of enzymes (Gao et al. 2020, Wu et al. 2020). Vithanage et al. (2018) found that biochar application increased the activity of redox enzymes, with significant increases in the activities of catalase and dehydrogenase but no significant changes in the activity of hydrolytic enzymes. The main reason for the increase in dehydrogenase activity is that the application of biochar increases the content of active organic matter in the soil, which in turn enhances the microbial activity in the soil and increases the activity of dehydrogenase mentioned conclusion is supported by the results of Gasco et al. (2016) and Bandara et al. (2017).

There is a close relationship between soil microorganisms and soil enzymes. The overall enzyme activity in soil consists of various intracellular and extracellular enzymes that originate from microorganisms (e.g., bacteria, fungi) or from plants and animals (Wang et al. 2015). Soil enzymes are also key factors affecting bacterial community structure. Xu et al. (2020) found that biochar application increased the activity of soil urease and the abundance of soil microorganisms, and they identified a significant correlation between the activity of urease and the abundance of soil microorganisms. There was also a close correlation ($P < 0.01$) between bacterial community structure and enzyme activity. Feng et al. (2021) found that catalase activity was significantly correlated with the relative abundance of soil communities of the phyla Gemmatimonadetes, Actinobacteria, Elusimicrobia, and Cyanobacteria and also identified catalase as a key factor influencing changes in the structure of soil bacterial communities. The direct effect of biochar on enzymes refers to the adsorption of enzymes or substrates by biochar, which in turn affects enzyme activity and properties, and is an important mechanism through which biochar regulates soil enzyme activity. The indirect effect of biochar on soil enzymes refers to the interaction between specific microorganisms and plant roots that is stimulated by biochar, thus affecting enzyme activity, expression, and secretion levels (Oleszczuk et al. 2014, Głodowska et al. 2017, Gorovtsov et al. 2020).

The mechanisms through which biochar affects soil enzymes and microorganisms need to be clarified for specific microorganisms and enzymes, as biochar stimulates specific microbial communities and thus affects the activity of their related enzymes. For example, biochar affects the fungal community, thus affecting the activity of ligninolytic enzymes. The research on the influence of biochar on soil enzymes and microorganisms is not sufficient, and research in this area should be further deepened.

Biochar reduces the toxicity of pollutants to microorganisms

Biochar has been applied during the remediation of contaminated soils in recent years, and its adsorption properties have been widely used in the remediation of heavy-metal-contaminated soils (Li

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et al. 2020). Igalavithana et al. (2017) found that biochar made from vegetable residues adsorbed Pb from the soil, reduced the harm caused by Pb to microorganisms, and increased the biomass of soil microorganisms and the enzyme activity. The application of biochar had similar effects in soils contaminated with a combination of Cd and Pb. Xu et al. (2018) found that the application of nutshell biochar reduced the concentrations of Pb and Cd, reduced the toxic effects of Pb and Cd on microbial communities, improved the carbon sequestration capacity of the soil, and reduced the emission of carbon dioxide from the soil environment to the atmosphere. Biochar can improve the remediation of Pb- and Cd-contaminated soil *via* adsorbing heavy metals and improving soil enzyme activity.

A large number of studies have been conducted on the combined effect of biochar and microorganisms on the remediation of contaminated soils. The combination of *Bacillus cereus* and *Pseudomonas japonica* with biochar was found to have a remediation effect on Cr-contaminated soils, and the combined effect of biochar and microorganisms was found to promote the conversion of Cr^{6+} to Cr^{3+} and reduce the toxic effect on plants (Arshad et al. 2017). The interaction between *Neorhizobium huautlense* and biochar stimulates the growth of crops and significantly reduces heavy metal contents in crops, which is effective in the remediation of Pb- and Cd-contaminated soils (Wang et al. 2016). The interaction between biochar and soil microorganisms in the soil influences the transformation of heavy metals, while the microorganisms colonise the biochar surface and absorb heavy metal ions from the soil solution, resulting in lower concentrations of heavy metals (Zhu et al. 2017).

Biochar and microorganisms are also commonly used together in the remediation of organic pollutants. The biochar-associated microflora QY1 (a novel microbial consortium) plays an important role in the biodegradation of polycyclic aromatic hydrocarbons (PAHs). The dominant genera in the QY1 flora are *Methylobacterium*, *Burkholderia*, and *Stenotrophomonas*, and the combination of these dominant genera and biochar results in a much higher degradation rate of phenanthrene (PHE) and pyrene (PYR) (Li et al. 2021). Biochar and microorganisms together also affected the expression of genes related to PAHs, and the proportions of Arthrobacter and Flavobacterium in the inter-rhizosphere microorganisms of rice (*Oryza sativa* L.) and carrot (*Daucus carota* L.) increased after the application of biochar to maise stems. The interaction of biochar with Arthrobacter and Flavobacterium promoted the degradation of PAHs and the expression of related genes in the soil (Ni et al. 2017, 2018). The degradation of organic pollutants caused by the interaction between biochar and soil microorganisms may be due to the interaction between hydrogen peroxide produced during the degradation of PAHs and persistent free radicals on the surface of biochar, which results in the formation of hydroxyl radicals that destroy the structure of PAHs and increase the effectiveness of microorganisms for degradation and transformation, thus accelerating the pollutant degradation process (Zhu et al. 2017, Sazykin et al. 2018). The degradation of soil organic pollutants is an extremely complex process that is influenced by many factors. The properties of biochar itself, soil characteristics, and microbial flora are all closely related to this process. Biochar should be tested for heavy metals and organic pollutants before its application, and potential biochar contamination with heavy metals and organic pollutants should be further explored in future studies.

Effect of biochar on microbial signalling molecules

N-Acyl homoserine lactone (AHL) is a signalling molecule that regulates inter-microbial behaviour. Biochar adsorbs AHL, thereby affecting gene expression and intraspecific communication in soil microorganisms, most commonly in gram-negative bacteria (Sun et al. 2021). The adsorption of signal molecules by biochar is related to its pyrolysis temperature, with biochar produced *via* high-temperature cracking having a higher capacity to adsorb signal molecules, as well as interfering more strongly with bacterial inter- and intra-specific signal communication (Masiello et al. 2013).

Biochar also affects the communication of microbial signalling molecules by adjusting the pH of the soil. The degree of hydrolysis of AHL, a signalling molecule that is more sensitive to changes in pH, increases with increasing pH, which reduces intercellular signalling by bacteria. However, farnesol, a signalling molecule that is not sensitive to changes in pH, has no significant effect on inter- and intra-species signalling by fungi (Gao et al. 2016, Zhu et al. 2017).

The effect of biochar on signalling molecules causes plants to respond to environmental stresses. Biochar application increased resistance to *Leveillula taurica*

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and *Botrytis cinerea* in tomatoes and pepper (Elad et al. 2010). The increase in crop resistance was related to the amount of biochar applied, with higher application rates increasing plant stress resistance to a greater extent compared to lower application rates (Elad et al. 2010). The type of biochar influences the interaction between plants and microorganisms (Meller et al. 2012). The effects of different types of biochar on arbuscular mycorrhizal fungi (AMF) and *Fusarium oxysporum* in the tomato root system were found to differ, with waste biochar not enhancing AMF colonisation in the plant root system and wood-prepared biochar affecting the root secretions of the plant, which in turn inhibited the growth and development of *Fusarium oxysporum* mycelium (Akhter et al. 2015). There are also differences in the contaminants contained in different types of biochar. The volatile organic compounds (VOCs) contained in biochar can inhibit microbial metabolism and may also be an uncertain factor influencing plant resistance (Spokas et al. 2011). To better understand the effect of biochar on signalling molecules, more research is needed on the quantification of biochar, pyrolysis temperature, and endogenous contaminants.

Potential harm to the soil environment due to biochar application

Although biochar has been shown to be beneficial to the soil environment, there are potential hazards associated with biochar. Biochar is produced *via* biomass pyrolysis in a fully or partially anoxic state. The material used to prepare biochar is usually crop straw, livestock waste, or urban sludge. The contaminants contained in these materials may enter the soil media with the application of biochar, thus posing a potential hazard to the soil environment (Devi and Sahora 2014). Biochar may contain heavy metals, organic pollutants, and other toxic and hazardous substances (Li et al. 2018) (Table 1). In addition, the concentration of heavy metals contained in the raw material determines the concentration of heavy metals in biochar. It has been found that the concentrations of Cd, Pb, and Zn in biochar vary with the pyrolysis temperature (Liu et al. 2016, Zhang et al. 2020). Therefore, it may be necessary to determine the optimal pyrolysis temperature range before using it.

In addition, the VOCs contained in biochar applied to soil can harm microorganisms. The presence of VOCs in biochar may depend on the pyrolysis temperature (300–600 °C) during biochar production; these compounds should be volatilised with increasing temperature, and some semi-volatilised organic compounds could be accumulated in biochar (Lian and Xing 2017). Sun et al. (2015) found that biochar application reduced the number of *Bacillus mucilaginosus* in the soil because of VOCs contained in the endogenous source of biochar that inhibited the growth of *Bacillus mucilaginosus*. PAHs are a group of toxic and hazardous organic pollutants produced during the incomplete combustion of biomass (Li et al. 2016), but with proper pyrolysis conditions, PAHs would be decreased in produced biochar (Lyu et al. 2016).

Table 1. Hazards of endogenous biochar pollutants to soil microorganisms

Pollutant	Examples	Hazards	Reference
Heavy metals	Pb, Cd, Zn	inhibits microbial growth, reduces biomass, affects microbial enzyme activity	Qin et al. (2021), Devi and Sahora (2014)
Organic contaminants	polycyclic aromatic hydrocarbons, volatile organic compounds	mutagenic effects on microorganisms, inhibition of microbial activity, alteration of microbial community structure	Anjum et al. (2014), Joner et al. (2001)
EPFRs	hydroxy, alkoxy	reduces enzyme activity in cells, causes oxidative stress, and affects the community structure of microorganisms	Odinga et al. (2020)
Other pollutants	perfluorinated compound, perfluorooctane sulphonate, pentadecafluorooctanoic acid	alters the diversity and abundance of soil bacterial communities, inhibits enzyme activity	Cai et al. (2020)

EPFRs – environmental persistent free radicals

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However, the effect of PAHs on soil microorganisms tends to be low because the adsorption capacity of biochar limits the entry of PAHs into the soil medium, and the effective concentration of PAHs in biochar is much lower than the maximum permissible concentration in the soil environment. This explains the low negative effect of endogenous contaminants in biochar on soil microorganisms (Wang et al. 2017, Xiang et al. 2021).

The pyrolysis of biochar generates not only pollutants, such as VOCs and PAHs but also a new type of environmental pollutant: environmental persistent free radicals (EPFRs). EPFRs are specific oxygen-containing radicals, such as hydroxyl, alkoxy, semi-quinone, and peroxy radicals, that are produced during the pyrolysis of biochar and transfer electrons to the transition metals (e.g., Fe, Cu, and Ni) in the biochar. EPFRs remain in a more stable state in systems formed by organic matter and particulate matter, resulting in the generation of a large number of EPFRs (Qin et al. 2018, 2021). EPFRs cause oxidative stress in plants, inhibit seed germination, and damage plant rootstocks. In addition, EPFRs induce oxidative reactions that lead to oxidative stress in soil microorganisms and cause damage to microbial communities (Qin et al. 2021). Odinga et al. (2020) showed that EPFRs in biochar could have a toxic effect on soil bacteria, reducing their activity and numbers and altering their community structure, which can inhibit the early germination stage of plants.

Summary and prospects

The key mechanisms through which biochar affects soil microorganisms after application to soil are as follows: (1) the pore surface structure of the biochar can provide a favourable habitat for microorganisms and can promote their growth; (2) biochar promotes the growth and reproduction of soil microorganisms *via* improving soil pH, water content, and aggregate content; (3) biochar provides sufficient nutrients for soil microorganisms; (4) the adsorption effect of biochar reduces the concentration and content of toxic and harmful substances in the soil, reducing the toxic effects of harmful substances on microorganisms; and (5) biochar affects microorganisms by influencing microbial signalling molecules.

Biochar shows good potential for tackling soil pollution and promoting agricultural development. Future research should explore the following: (1) the potential mechanisms of biochar-soil microbial interactions to gain further insight into the direct effects of biochar application on soil microbes; (2) the impacts of biochar on specific soil microbial communities, such as archaea, yeasts, protozoa, and other microorganisms that have a significant impact on biogeochemical soil cycles; (3) criteria for evaluating the impact of biochar on soil microbial diversity and function to overcome the difficulties in judging this impact; and (4) the response of soil microorganisms to biochar functional groups and the identification and application of specific biochar functional groups, which can clarify the deeper mechanisms of biochar effects on soil microorganisms.

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