

REVIEW

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# Microbial responses towards biochar application in potentially toxic element (PTE) contaminated soil: a critical review on effects and potential mechanisms

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## Abstract

Soil harbors a huge diversity of microorganisms and serves as the ecological and social foundation of human civilization. Hence, soil health management is of utmost and consistent importance, aligning with the United Nations Sustainable Development Goals. One of the most hazardous contaminants in soil matrix is potentially toxic elements (PTEs), which can cause stress in soil indigenous microorganisms and severely jeopardize soil health. Biochar technology has emerged as a promising means to alleviate PTE toxicity and benefit soil health management. Current literature has broadly integrated knowledge about the potential consequences of biochar-amended soil but has focused more on the physical and chemical responses of the soil system than microbiological attributes. In consideration of the indispensable roles of soil microbes, this paper first introduces PTE-induced stresses on soil microbes and then proposes the mechanisms of biochar's effects on soil microbes. Finally, microbial responses including variations in abundance, interspecific relationships, community composition and biological functions in biochar-amended soil are critically reviewed. This review thus aims to provide a comprehensive scientific view on the effect of biochar on soil microbiological health and its management.

## Highlights

- Sketched a mechanistic overview of PTE-induced stress towards soil microorganisms.
- Demonstrated a synopsis of biochar impacts on soil microbiota from both direct and indirect pathways.
- Discussed the development prospects of biochar technology aiming for a healthier agro-ecosystem.

**Keywords** Soil microbiological health, Biogeochemistry, Potential toxic elements, Biochar, Environmental impact assessment

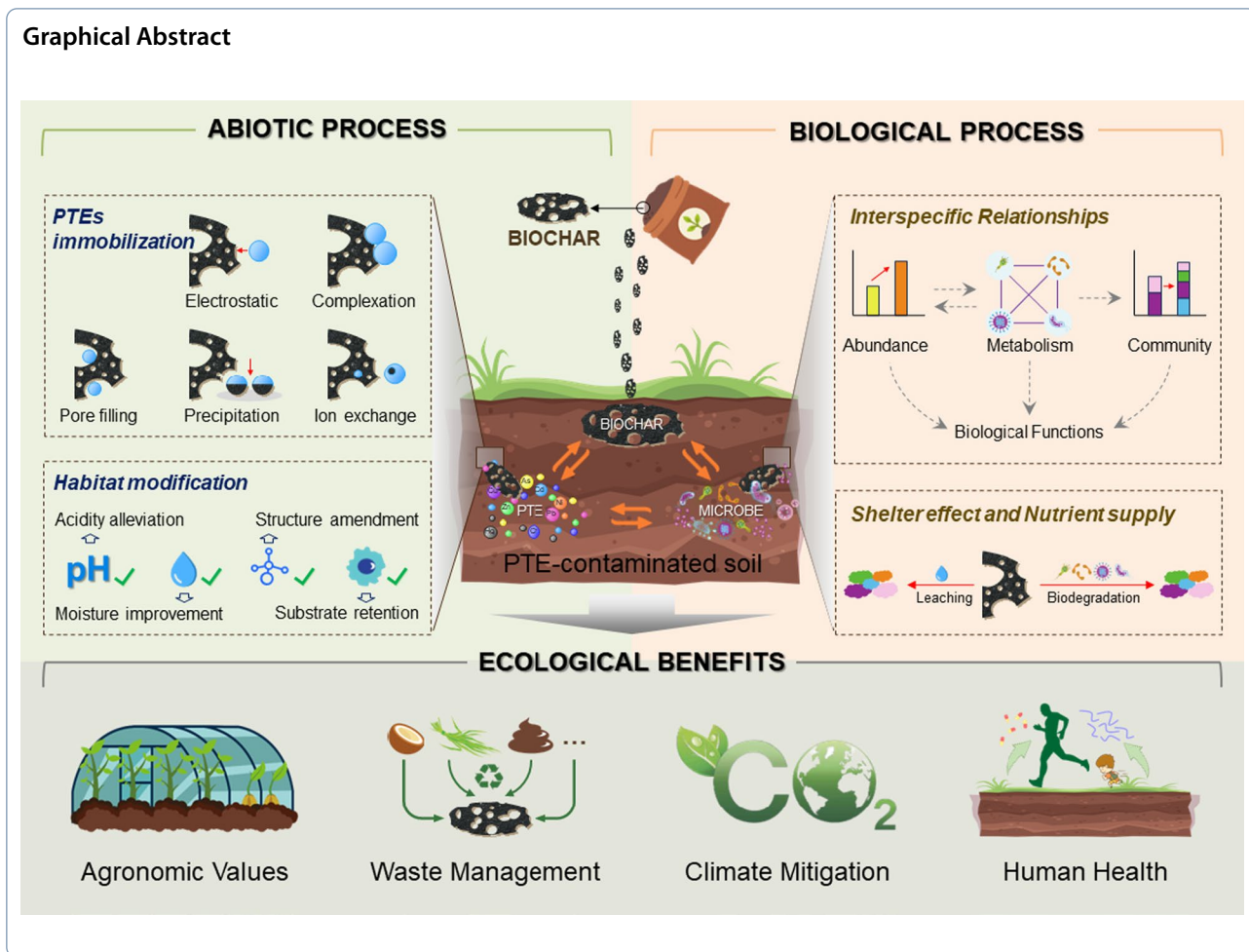
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### 1 Introduction

Soil quality is the historic origin of the current term “soil health”, whose scope extends beyond human health and highlights a broader sustainable goal on the planetary scale (Lehmann et al. 2020). Excessively accumulated potentially toxic elements (PTE, metals/metalloids show toxic or ecotoxic properties such as Cd, Pb, As etc.) severely jeopardize the soil health by aggravating soil structure destruction (Lwin et al. 2018), soil acidification (Dai et al. 2017), and fertility loss (Mansoor et al. 2021). The indiscriminate use of chemical pesticides and fertilizers (Sharma et al. 2021) and sewage irrigation (Murtaza et al. 2022) result in soil PTE pollution and menace the food security, thereby bringing a burden to the human health and ecosystem. Soil microbiological health substantially contributes to the soil health because microbes participate in various biogeochemical cycles and correlate with soil nutrient transformations (Mann et al. 2019). PTEs cause risks to soil microbes by altering their habitats and causing intracellular oxidative stress, resulting in a

decrease in microbial population and diversity, community composition variation, and functional deficiency (Tang et al. 2019), ultimately impairing the soil system’s ecological functions and social benefits (Lin et al. 2019).

Currently, a certain amount of PTE-contaminated soils is still used for crop cultivation due to the dearth of arable land, especially in Asia (Wang et al. 2021a, b), and such situation would presumably continue for a long period. In 2014, a joint report issued by the Chinese Ministry of Environmental Protection and the Ministry of Land and Resources showed that 15.6% of agricultural fields in China were contaminated by PTE (Zhao et al. 2015). In 2018, the Chinese Ministry of Ecological Environment implemented the standard of risk control for soil contamination, aiming to achieve the safe use of farmland mainly through periodic application of soil amendments (Wang et al. 2022). Many researchers have investigated effects of soil amendments on mobility and toxicity of soil PTEs (Mehmood et al. 2019), but broader understanding of the impacts

and consequences (e.g., microbial responses and soil enzymatic activities) in the amended soil is essential but still remains to be explored (Yu et al. 2022).

Biochar, referring to a carbon-rich product originating from pyrolyzed biowaste, is widely recognized as a sustainable and potent soil amendment for PTE immobilization and de-toxification (Zhang et al. 2019; Allende et al. 2023). Biochar can ameliorate soil conditions (e.g., water and nutrient retention, pH, soil texture) (Razzaghi et al. 2020) and revive soil microbial activity and soil functions (Palansooriya et al. 2019). Meier et al. (2017) demonstrated that biochar reduces Cu mobility through increasing soil pH, adsorption, and surface precipitation, which improved microbial abundance and soil basal respiration. Moreover, microbial metabolic activities and functions, including carbon use efficacy and fixation, were substantially rejuvenated due to the de-toxification of PTEs by biochar (Wang et al. 2020). Besides, biochar was evidenced to physically harbor soil microbials (Han et al. 2016), and simultaneously enhanced microbial resistance to combat PTE stress (Zhu et al. 2017), which was beneficial to soil microbiological health (He et al. 2021).

However, the current scientific understanding on the interaction of biochar and soil microbiological health in presence of PTEs is fragmented even non-consistent in some cases, which is neither systematic nor comprehensive. Lehmann et al. (2011) specifically reviewed the effect of biochar on soil biota, elucidating the potential interaction in the “soil-soil biota-biochar” system. Zhu et al. (2017) linked the microbial-induced enzymatic activities with biochar-induced changes of soil properties and summarized the biochar-microbial interaction on soil improvement and PTE mitigation. Moreover, Palansooriya et al. (2019) discussed the potential role of biochar on microbial activities (e.g., soil carbon mineralization, nutrient and enzyme activities) and proposed the key factors (e.g., chemical properties especially pH and soil organic matter (SOM), and physical properties such as pore size, pore volume, and specific surface area) determining the efficacy of biochar on microbial performance. While their priorities and disciplines differ, there is still room to comprehensively revisiting the effects of biochar application on soil microbiota. Notably our review is based on the “soil-biochar-PTE-soil microbials” system, summarizing the positive, negative, and controversial roles of biochar on soil microbials. We also underlined the direct, indirect, and potential effects and mechanisms by which biochar affects microbiological quality of the soil. Through a cross-sectional summary of the existed investigations, this review aims to promote follow-up studies to shed lights on potential mechanisms of biochar-induced microbial resilience in PTE-contaminated soils, especially from the perspective of soil health

and sustainability after biochar application. Therefore, we discuss that: (1) the consequences and mechanisms of PTE-induced stress on soil microbials; (2) the effect of biochar on PTE toxicity mitigation and soil microbial restoration; and (3) microbial responses to biochar application in PTE-contaminated soils. This work attempts to establish a theoretical basis and practical guidance to comprehensively understand how biochar acts on soil microbiological health management.

## 2 PTE-induced stress on soil microbials

The bioavailability and toxicity of PTEs are species-dependent (Lemire et al. 2013). Generally, organisms absorbing PTEs is a three-stage stepwise process, including (1) bio-accessibility, referring to the amount of PTEs that may be mobilized in soil matrix, (2) environmental bioavailability, denoting the amount of PTEs that may be accessible by soil organisms, and (3) toxic bioavailability, representing the amount of PTEs that cause adverse effects to targeted organs. Due to their non-degradable property, PTE contamination would take decades to have an effect. This sentence is confusing.

PTE contamination can lead to changes in soil physical and chemical conditions, in the dynamically irreversible and/or chronic manner. PTE-induced stress on soil microbials includes the deterioration of microbial habitat as well as intracellular oxidative stress. Table 1 summarizes the target sites, toxic mechanisms, consequences, and microbial resistance mechanisms towards PTEs. In this section, we focus on the inhibiting and lethal mechanisms of PTEs towards soil microbials.

### 2.1 Habitat deterioration

Due to the high sensitivity of soil microbials towards ambient habitat, effects of PTEs on soil microbials may be magnified. PTEs, such as Cr, Cu, Cd, and Pb, with strong hydrolysis capacity and high ionic charge, may cause soil acidification, especially in soil with limited buffering capacity (Bakshi et al. 2018; Liu et al. 2022a, b). PTE-induced inhibition of SOM mineralization and nutrient cycling may further cause substrate depletion and detrimental substance accumulation (Aponte et al. 2020). Nevertheless, the bioavailability of some plant essential elements (e.g., Fe, Zn, Ca, Mg) may decrease in PTE-contaminated soils (Kasowska et al. 2018).

Microbial habitats and environmental changes are closely associated with indigenous microbial characteristics, such as morphology, colonization, metabolism, activity, community composition and structure (Gourmelon et al. 2016). Jiang et al. (2019) analyzed the microbial characteristics of an e-waste site severely contaminated by Cu, Pb and Zn. The multivariate regression tree (MRT, 63%) results revealed that, rather than metals,

**Table 1** Toxicity mechanisms and consequences of PTEs to microorganisms and microbial resistance mechanisms

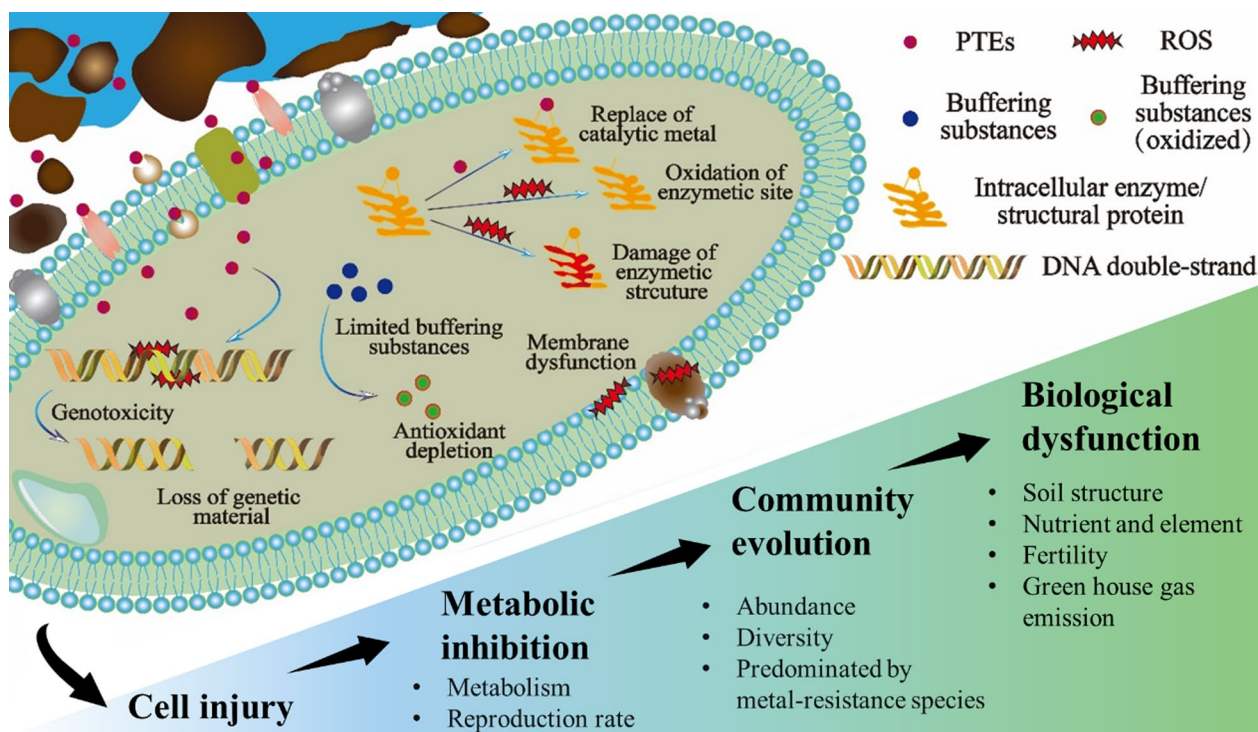
PTE	Target site	Toxicity mechanism	Consequence	Resistance mechanism	References
Hg	DNA, mRNA, protein	Destruction of Fe-S cluster of enzymes, indirectly inducing Fe release and generating ROS	Impact of oxidative phosphorylation and membrane permeability, inhibition of transcription, translation, cell division	Enzyme-based cleavage of C-Hg bonds and reduction, precipitation due to the generation of H <sub>2</sub> S	Roane et al. (2015); Xu and Imlay (2012)
As	Active sites of intracellular protein and nucleic acids	As (V): Substitution for phosphate due to the structural analogue As (III); Reaction with sulfhydryl groups of protein	Inference with P metabolism and P-dependent process, intracellular protein inactivation and dysfunction	Extracellular sequestration, phosphate uptake increase, chelation	Pandey et al. (2015)
Al	DNA, intracellular protein	Membrane damage and subsequent cytoplasmic aggregation	Inhibition of gene expression and enzyme synthesis, protein structure destruction	Extracellular immobilization (EPS), efflux pumps, formation of siderophore-metal complex	Jaiswal et al. (2018); Yaganza et al. (2004)
Cu	Cytoplasm	ROS generation via a Fenton-like reaction, due to the high affinity for peptides and SH-groups	Time-dependent toxicity, oxidative stress, cell membrane disruption, enzyme inactivation	Reduction, precipitation, chelation	Asatiani et al. (2021)
Cd	DNA, mRNA, active sites of protein and cell membrane	Outcompete with catalytic zinc, nonspecifically binding with DNA	Enzyme inactivation, single-strand break	Precipitation, efflux pumps, EPS sequestration, cell wall or outer membrane binding	Roane et al. (2015)
Pb	Functional groups of membrane and proteins, proteins and nucleic acid	Complexation with sulfhydryl groups, binding with hydroxyl and phosphate groups of nucleic acid	Inhibition of cell division and respiration, protein denaturation, disruption of cell membrane, DNA damage	Biosorption by cell wall, extracellular sequestration, chelation, efflux pumps	Kushwaha et al. (2018)
Fe	DNA, intracellular antioxidant, active sites of protein	ROS generation due to the oxidative nature of Fe(III)	Oxidative stress and hence damage of proteins and nucleic acids, antioxidant depletion	Chelation via siderophores, reduction, efflux pump	Lemire et al. (2013); Schalk et al. (2011)
Ni	DNA, lipid, protein	Substitution of essential metal of metalloproteins, binding with essential metal of metalloproteins or outside of the catalytic site, ROS generation	Enzyme inactivation and dysfunction, DNA, lipid denaturation	Efflux pump, vacuolar sequestration	Macomber and Hausinger (2011)

soil texture (31%) and organic carbon (OC,14%) were the main variables influencing bacterial taxonomic composition; however, soil pH (23%) and soil texture (14%) were the main variables influencing microbial diversity (Jiang et al. 2019). Variations in soil properties in turn would influence PTE mobility and speciation transformation, as previous researches have suggested (Tang et al. 2019). The substitution of predominant microbial species, and evolution of microbial community driven by PTE-induced changes in nutrient availability have been used to explain the adverse effects of PTEs on microbials (Wu et al. 2018).

### 2.2 Cell injury

Bioavailable PTEs can penetrate the cytoplasm and access intracellular target sites such as DNA and proteins, causing molecular toxicity including membrane integrity damage, intracellular enzyme abolishment, antioxidant substance depletion, and intracellular component (e.g., lipid, DNA) oxidation and destruction (Abdu et al. 2017; Robins et al. 2022) (Fig. 1). Generally, the above effect can be interpreted as the intense affinity between certain metal species and biological macromolecules such as mercapto protein, nucleic acid bases and phosphate (Chu 2018).

Literature has demonstrated that PTEs such as Fe and Cu may promote electron transfer, which favors the formation of reactive oxygen species (e.g., ROS, including  $\cdot\text{OH}$ ,  $\text{H}_2\text{O}_2$ ,  $\text{O}_2^{2-}$ ) via Fenton and Fenton-like reactions (Huang et al. 2020). Wu et al. (2020) reported that the bio-oxidation activity of acidophilic bacteria was inhibited by high levels of  $\text{Co}^{2+}$ , resulting in intracellular antioxidant substance depletion, homeostasis imbalance and dysfunction. PTEs may substitute or react with surface functional groups of enzymes. For example, As can severely inhibit the P-dependent process. As(III) can substitute for phosphate due to their structural similarities, while As(V) can react with sulfhydryl groups (Huang et al. 2020). Combination of PTEs and signal receptors on cell membrane can disrupt intracellular information transmission (Abdu et al. 2017). In addition, PTEs, especially Ag, can damage the bacterial electron transfer chain and pose genotoxicity towards microbial cells by the metal-mediated Fenton chemistry, resulting in DNA damage and mutation (Chu 2018). Moreover, oxidation of the amino acid chains may trigger protein degradation and enzyme defunction, hampering the intracellular homeostasis (Li et al. 2021). PTE-induced intracellular toxicity can sometimes be acute. Sumner et al. (2005) proposed that the protein carbonyl levels of *S. cerevisiae*



**Fig. 1** PTE induced stress towards soil microorganisms. Bioavailable PTEs can induce intracellular oxidative stress mainly through (1) generating ROS, (2) destructing enzymes and cell membrane, and (3) oxidating DNA, lipids, causing individual cell injury, which lead to metabolic inhibition. From the temporal perspective, PTE-induced stress may further drive the evolution of primary community and lead to biological dysfunction

were sharply increased within several minutes when exposed to 0.5 mM Cr(VI) liquid. PTE-induced cell injury may worsen cell apoptosis and reduce microbial biomass, jeopardizing soil microbiota.

### 2.3 Metabolic inhibition

Intracellular dysfunction as well as gene expression abnormalities may possibly suppress microbial activity of PTE-contaminated soils at a relatively low level (Al-Wabel et al. 2019). Microorganisms may distribute more energy to basic function maintenance rather than cell proliferation under oxidative stress, resulting in starvation-caused growth retardation (Zhao et al. 2022). The inferior microbial activity may lead to the loss of C storage of topsoil, and hence increase soil C emission (Zahorec et al. 2022). Some researchers believed that microbial growth would occur only when bioavailable PTEs were reduced to a threshold concentration (Yao et al. 2020). Microbial enzyme deletion may affect soil fertility due to the repression of SOM turnover. A meta-analysis indicated that PTEs led to changes of soil total nitrogen (TN) and OC by  $-17.9\%$  and  $-4.95\%$  (Zhou et al. 2016). Meanwhile, the newly formed SOM in PTE-contaminated soil could be allocated to the labile fraction due to the absence of soil microorganisms (Zahorec et al. 2022).

However, soil microorganisms have developed a variety of resistance mechanisms towards PTEs due to the long-term exposure. Microbial resistance towards PTEs may be discrepantly subject to biochemical and morphological characteristics of specific microbial species, distribution of metals in cellular fractions (e.g., membrane, cytoplasm, nucleus and nucleoid), and stress reactions of soil microorganisms (Prabhakaran et al. 2016). Some soil microorganisms could temporarily evade PTE-induced stress through dormancy, whereas metal-tolerant species increase their population when exposed to PTEs (Mann et al. 2019).

### 3 Benefits of biochar application for soil microbials

Biochar (1) immobilizes, and hence reduces toxicity of PTEs (O'Connor et al. 2018), (2) modifies soil microhabitat, (3) maintains overall soil nutrient level and availability, and (4) potentially serves as shelter for soil microbials by ameliorating PTE-induced stress and conserving soil microbials (Blanco-Canqui 2021; Hagemann et al. 2017). The effect of biochar on soil microbial in PTE-contaminated soils is summarized in Fig. 2.

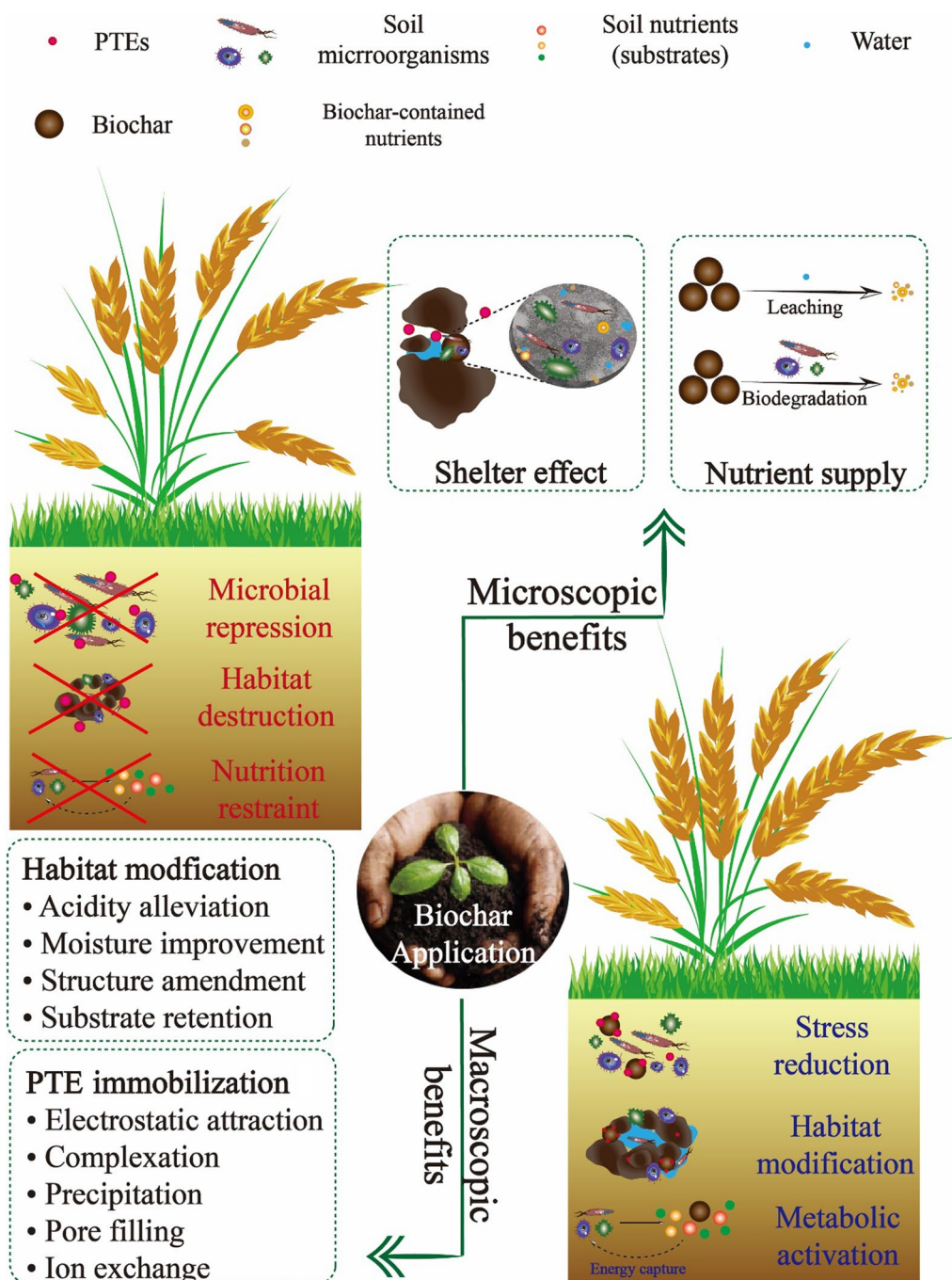
### 3.1 Minimizing PTE toxicity

#### 3.1.1 Immobilization

PTE immobilization refers to the redistribution of metal speciation, namely transformation from bioavailable forms into non-bioavailable forms (Guo et al. 2017). Generally, biochar can immobilize PTEs through electrostatic attraction, cation exchange, complexation, and precipitation. Specific immobilization mechanism varies with variables such as biochar properties (e.g., micropores proportion and distribution, surface chemistry), metal species, and soil conditions (e.g., pH, SOM, minerals) (Wang et al. 2021a, b).

Biochar interacts with PTEs through (1) complexation (e.g.,  $\text{Hg}^{2+}$ ), (2) surface precipitation (e.g.,  $\text{Cu}^{2+}$ ) or reduction-precipitation (e.g.,  $\text{Cr}^{6+}$ ), (3) pore filling, (4) ion exchange (e.g.,  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ) and (5) electrostatic attraction (e.g.,  $\text{As}^{3+}$ ) (Bandara et al. 2020). The diffuse outer-sphere of biochar-PTE compound is mainly composed of hydrated metal ions, which is highly leachable (Chauhan et al. 2023; Lian and Xing 2017). However, biochar's electronegative surface to some extent facilitates the formation of the inner-sphere bound (Liang et al. 2021). Dehydrated PTEs can react with surface functional groups of biochar (e.g., hydroxyl, carboxyl, phosphoryl), physically adsorbed through ion exchange or pore filling, or chemically adsorbed through complexation (Qiu et al. 2022). The generation of binary, ternary, or annular chelates through poly-coordination reduces environmental bioavailability of PTEs, due to the abundant coordinating groups and three-dimensional porous structure of biochar. PTEs may react with inorganic minerals (e.g., carbonate, sulphate, phosphate and hydroxide) and thus precipitate on biochar surfaces (Wu et al. 2021). Furthermore, as PTEs enter surface micropores, biochar matrix swells to accommodate more sorbates, enlarging pore volumes and potentially causing biochar matrix collapse (Lian and Xing 2017). This explains the desorption hysteresis and low durability when immobilizes PTEs.

Soil As is primarily found in the form of arsenite and arsenate, which may escape from biochar immobilization and be mobilized due to pH rise (Huang et al. 2020). Several modification methods, such as surface modification and composites with other functional materials, have alleviated the dilemma and exhibited superior performance in As decontamination (Vithanage et al. 2017). In general, total immobilization capacity of biochar is predominantly driven by surface chemistry rather than non-specific adsorption



**Fig. 2** Amelioration of PTE-induced stress by biochar in agricultural soil. Biochar application can alleviate PTE-induced stress through macroscopic mechanisms such as (1) PTE immobilization and (2) habitat modification, and microscopic mechanisms comprising shelter provision and nutrient supply. The rejuvenated soil microbiota because of biochar application can be of immense agricultural values

(Arabyarmohammadi et al. 2017). Given the significant role of biochar’s surface functionalities (El-Naggar et al. 2021), we need to recognize the effects of micropore porosity and distribution on PTE immobilization, and more investigations should be carried out in the

field of micro-precise regulation of biochar surface functionalities.

### 3.1.2 Sequestration

The term “immobilization” emphasizes the effect of soil amendment, whereas “soil sequestration” refers to

combination of diffusion limitation, adsorption, and partitioning of PTEs in soil matrix (Shuai et al. 2021). Except for surface functionalities and chemistry, biochar-induced changes in the abundance of soil critical ingredients involving minerals, SOM and microbial secretions are closely associated with the fractionation, distribution, mobility of PTEs (El-Naggar et al. 2021). Biochar application can affect a series of soil physical, chemical, and biochemical processes, resulting in changes in soil properties, and accelerating the transformation of PTEs to non-bioavailable phases. Four main mechanisms are discussed as follow.

Firstly, the alkaline nature of biochar expedites the transformation of metals from soluble, exchangeable, non-specific adsorbed forms into hydroxyl-complexed phases ascribed to the liming effect (Wang et al. 2019a, b). The formed metal hydroxyl complexes further facilitate surface adsorption of soil particles due to the decreased ion-solvation interaction. In a 7-year field experiment, Wang et al. (2021a, b) found that bioavailable Cd, Pb, Zn, and Mn were significantly reduced by increased soil pH by biochar. Secondly, soil aggregates, which refer to the complex compound of soil minerals, SOM, microorganisms, and their extracellular polymeric substances, are considered as natural adsorbents for PTEs. Therefore, biochar-induced co-fluctuation of soil colloids (e.g., microaggregates, SOM), which primarily occur in the saturated zone, can restrain the toxicity of water-soluble metals (Liu et al. 2022a, b). Variables such as the frequency of turnover and soil leaching, are directly associated with soil aggregate stability and PTE bioavailability. Thirdly, biochar increases soil OC that such fraction contains amounts of low molecular weight organics with strong affinity towards PTEs and thus indirectly influences mobility of PTE (Andreas et al. 2016). However, biochar itself may also serve as a source of PTEs due to its feedstock and pyrogenic conditions (Hameed et al. 2019). The endogenous release of PTE from biochar is an intricate process, which is associated with the volatile matter content and acid functional group density of biochar, pH and ionic strength of soil solution (Hameed et al. 2019). Finally, biochar-induced changes in soil trace elements involving S, Al, Fe and Mn may relate to PTE immobilization. El-Naggar et al. (2021) demonstrated the positive correlation between soil V and both S and Al, which was explained as the S chemistry and degradation of Al-containing complexes.

### 3.2 Promoting the microhabitat

The term 'biochar' was proposed by the inspiration of Terra Preta, a highly fertile anthropogenic soil in

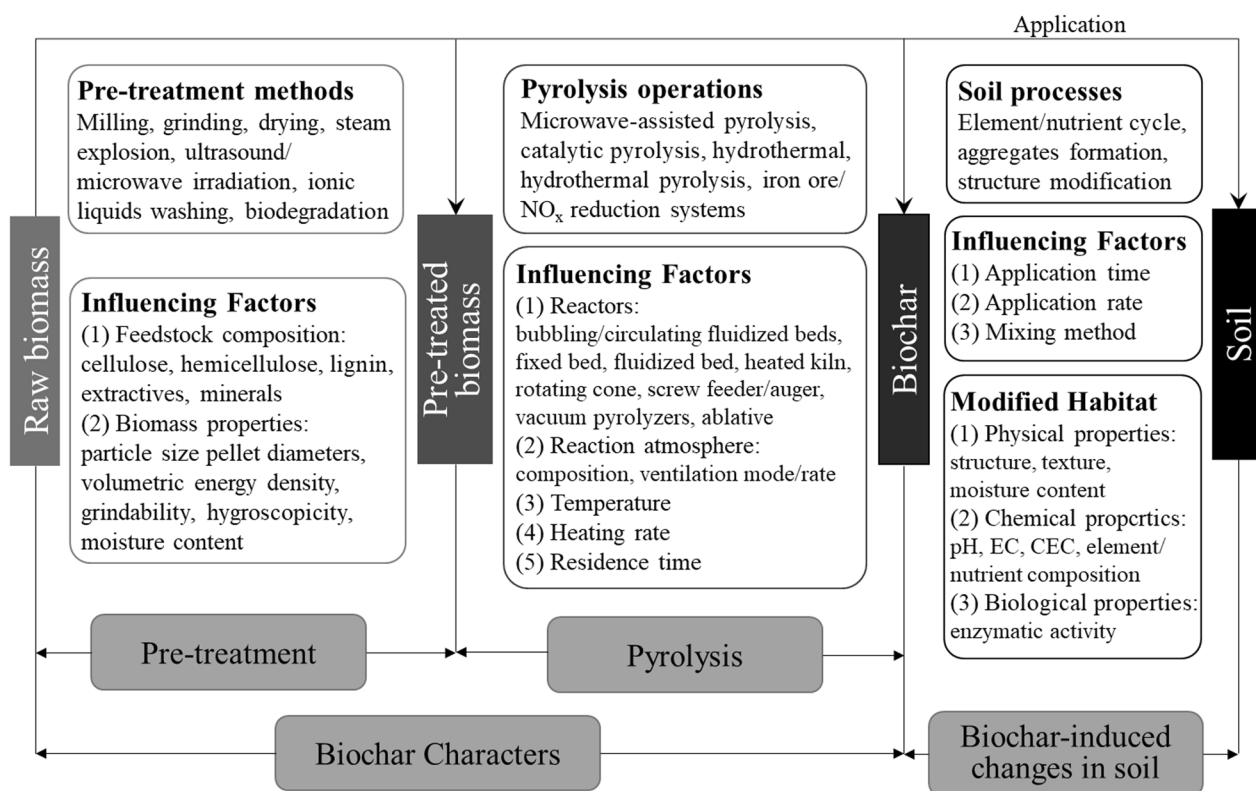
the pre-Columbian Amazon (Bezerra et al. 2019). In the context of ecological modernization, biochar is thought to offer various improvements to agricultural soil (Yuan et al. 2019). Consequently, the modified soil conditions may in turn serve as a favorable microhabitat for indigenous microorganisms.

#### 3.2.1 Soil environment quality

Application of biochar induced changes in soil pH, electrical conductivity (EC), texture, and moisture content that may conjointly modify soil environment quality, which is critical for the diversity and abundance of soil bacteria (Nguyen et al. 2018). The presence of negatively charged functional groups in biochar surface (e.g., hydroxyl, carboxyl, and phenolic groups), and minerals (e.g., carbonates, bicarbonates, silicates) can bind with  $H^+$  in soil solutions, contributing principally to the increase of soil pH to the increase of soil pH (Gul et al. 2015). The elevated soil pH may lead to increase of microbial biomass due to the stimulatory effect. Chen et al. (2017) investigated the short-term microbial responses to fine particle biochar addition. They found that the concentrations of total microbial phospholipid fatty acids (PLFAs, indicator of microbial biomass) were increased due to the modified soil texture, increase of soil pH and EC. Liu et al. (2017) illustrated enhanced  $N_2$  emissions when biochar increased soil moisture to 70% of full capacity, which was explained as the reactivation of anaerobic denitrifying bacteria. Domene et al. (2014) certified the strong positive correlation between soil moisture and microbial biomass in biochar-amended soils. Accordingly, beneficial effect of biochar is more obvious in drought regions (Paetsch et al. 2018). Biochar-induced benefits in soil microbiota can mainly be ascribed to component composition and physicochemical structures. Influencing factors are comprehensively summarized in Fig. 3.

Current investigations indicated soil bacteria and fungi played a critical role in determining soil stability and resistance towards PTE contamination (Khan et al. 2019; Njoku et al. 2020). Soil bacteria and fungi activity is highly sensitive to environmental changes; therefore, increases in soil pH and mineral elements, inorganic and organic compounds may benefit soil fungi (Nie et al. 2018). As for the arbuscular mycorrhizal fungal (AMF)-plant symbiont, addition of biochar could result in a higher colonization rate of the host plant roots due to the modification of soil texture (Rummel et al. 2017). Meanwhile, enhanced soil resistance against PTEs leads to augmented dehydrogenase and urease activities and improved microorganism colonization (Ji et al. 2022). The porous structure of biochar improved both air permeability and water retention, therefore boosting





**Fig. 3** Factors associated with biochar-induced microbial responses. In PTE-contaminated soils, microbial responses are conjointly influenced by biochar characters (e.g., PTE immobilization, colonization, nutrient supply) and biochar-induced changes in soil (e.g., habitat modification, enzyme activity restoration)

physiological activity and benefiting hyphal growth. In addition, the augmentation of soil bacteria in biochar-amended soil is beneficial for reducing the PTE bioavailability by accelerating extracellular electron transfer (Zheng et al. 2021).

### 3.2.2 Physical isolation

Biochar can serve as a potential habitat for soil microorganisms mainly due to its large surface area, porous structure and adsorption capacity, physically isolating the colonizing microorganisms from PTEs and predators (Ennis et al. 2012). In addition, water molecules and essential substrates (e.g., alcohols, aldehydes, ketone and carbon sources) may be preserved in mesopores and micropores (Johannes et al. 2015), providing essential substrates for soil microorganisms.

Pietikäinen et al. (2000) creatively prepared a ‘microcosms’ to evaluate the effect of biochar on the capture of organic compounds and metabolic activity of microorganisms. The result showed that biochar harbored a small fraction of microbial biomass but nearly possessed more than an order of magnitude in reproduction rate when

compared to the control experiment. Micropore distribution, size, and the surface roughness, topography, surface charge and hydrophobicity of biochar conjointly influence microbial attachment and colonization (Rummel et al. 2017). Recently, the possibility of long-term microbial colonization has been researched. Several strains colonized in poplar wood biochar were observed to form biofilms on plants roots (Bertola et al. 2019). Furthermore, biochar could serve as proper carrier for engineering strains for alleviating their sensibility against the PTE stresses (Siddeeg et al. 2019; Ma et al. 2020). It remains to be seen whether biochar can serve as ecological niches for soil microbials, which is primarily dependent on the growing conditions of microbial species and habitats.

### 3.2.3 Nutrient supply

Domene et al. (2014) proposed that most impacts of biochar on soil microorganisms were attributed to the changes of nutrient availability. As an organic amendment, biochar improves soil nutrient availability and retention by supplementing the soil with P, K, S, and other trace minerals. Soil microorganisms can utilize the soluble nutrients contained in biochar, and liable

fraction through bio-fragmentation, bio-assimilation and bio-mineralization based on co-metabolism (Karim et al. 2020).

Biochar can alter elemental composition of the microhabitat and influence microbial metabolic activity (Song et al. 2018). The improved proportion of C substrate may promote microbial catabolism and thus accelerate SOM mineralization (Wei et al. 2020). The following variations in SOC cycling may result in changes in bioavailability and transformation of other nutrients, which were considered as the priming effect of biochar (Karimi et al. 2020). Besides, content and bioavailability of the biochar-released C, N, P and K may be distinct due to the heterogeneity of various biochar products (Al-Wabel et al. 2019). In calcareous soils with low nutrient availability, Karimi et al. (2020) reported that application of corn residue biochar enhanced the bioavailability of soil inorganic N and some trace elements (e.g., Fe, Zn, Cu), therefore promoting the microbiological processes. Notably, this may be a long-term effect because of the slow-release property of biochar product, which maintained nutrient availability on long-time scale (Jiang et al. 2019). Additionally, the fungal colonization rate was significantly increased due to the biochar-induced increase on soil N/P ratio.

Biochar may also immobilize exogenous bioavailable nutrients in some cases. Taghizadeh et al. (2012) claimed that biochar-immobilized ammonia was relatively bioavailable in soil through isotopic analysis. Moreover, biochar exhibited better microbiological value rather than certain nutrients for bio-stimulation. Xu et al. (2018) pointed out that total microbial metabolic activity in biochar-amended soil was as 1.25 times as glucose-amended groups. This showed the potential of biochar on the improvement of microbial activity in nutrient-limited soil matrix.

Biochar-induced changes in element and nutrient availability were considered as a dynamic equilibrium between adsorption and release, which was feedstock dependent and thus remains controversial. Biochar induced reduction in element and nutrient availability was also reported (Muhammad et al. 2016). Previous studies focused on the links between biochar characteristics and adsorption capacities, and investigating the specific sorption capacity of biochar is conducive for diminishing the uncertainty of biochar application.

#### 4 Microbial responses in biochar-amended soil

Periodic biochar application, as the impulsive influence towards soil microbials, may change both the rate and direction of microbial evolution during a long period. Rather than the short-lived abiotic changes, biochar-initiated changes to soil microbiota may be amplified

over time (Hol et al. 2017). Based on the highly sensitive nature of soil microbiota, the responses primarily comprise changes in microbial abundance, interspecific relationships, community composition and structure, and biological functions. Table 2 summarizes the microbial responses towards biochar application in PTE-contaminated soils.

##### 4.1 Microbial abundance

Generally, biochar addition stimulates microbial activity and thus increases microbial abundance in PTE-contaminated soils. Alleviation of PTE toxicity, modification of microbial habitat, sheltering effect, and the improvement of soil nutrient availability (Fig. 3) collectively contribute to the changes in microbial reproduction rate and abundance. With respect to the ignorable interspecific difference (e.g., optimum growth conditions, resistance, and resilience towards environmental changes), any of the biochar-induced changes may differ and even lead to controversial consequences.

Reduction of PTE toxicity is of vital significance to sustain soil microbiological health. Moore et al. (2018) demonstrated that adding chicken manure biochar (5% w/w) enhanced microbial abundance and soil basal respiration due to the reduction of exchangeable Cu. Besides, physically isolated by biochar, the colonized microorganisms were endowed with superior metabolic and reproduction rate. Moreover, the positive effect of biochar on early growth stage of soil microorganisms has been often found. For instance, biochar derived from beet root chips significantly improved spore germination of AMF with 10 vol% addition (Rillig et al. 2010). It further facilitated the root colonization and fungal inoculum formation, and hence enhanced nutrient delivery of AMF to plant, which in turn stimulated the activity of the plant-AMF symbiosis. Recently, Xu et al. (2021) conducted a global meta-analysis using structural equation modeling and found that the fungal abundance was an important factor influencing bacterial abundance in biochar-amended soils, indicating the limiting effect of interspecific relationships.

However, biochar may pose hazards to soil microbiota. Biochar itself may be a source of organic and inorganic contaminants, such as PTEs, polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs) and dioxins (Godlewska et al. 2021). This is highly feedstock dependent, and may also results from improper pyrolysis (Hilber et al. 2017). Both anthropogenic and manipulative factors are organized in Fig. 4. As seen, the misuse of biochar would cause negative consequences for soil microbials. A comprehensive consideration is essential before the practical application of biochar. However, Hol et al. (2017) hypothesized biochar addition may suppress

**Table 2** Microbial responses towards biochar application in PTE-contaminated soils

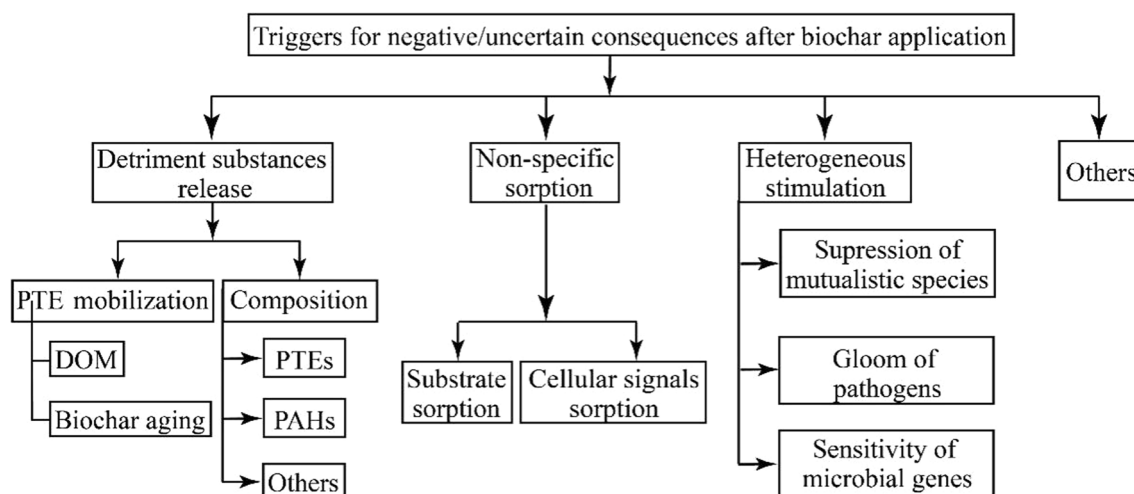
Contamination level	Biochar characteristics		Variation of soil conditions		Microbial response		References
	Feedstock	Pyrolysis treatment	Application rate	Abundance and diversity	Dominant species	Biological function	
Pb (1945 mg·kg <sup>-1</sup> ) As (171 mg·kg <sup>-1</sup> )	Pine needles	300 °C, 7 °C·min <sup>-1</sup> , 3 h	10% by weight	AI, DD (especially towards bacteria)	Gram bacteria, Actinomycetes, arbuscular mycorrhizal fungi	–	Ahmad et al. (2016)
Pb (87 mg·kg <sup>-1</sup> ) Cr (114 mg·kg <sup>-1</sup> ) Ni (26 mg·kg <sup>-1</sup> ) Cu (38 mg·kg <sup>-1</sup> ) Zn (1578 mg·kg <sup>-1</sup> )	Wine lees	600 °C, 5 °C·min <sup>-1</sup> , 2 h	1% by weight	AI, DI	Actinobacteria, Firmicutes, Proteobacteria, Cyanobacteria	Enhancing N <sub>2</sub> fixation and C cycling	Xu et al. (2017)
As (55 mg·kg <sup>-1</sup> ) Cu (50 mg·kg <sup>-1</sup> ) Zn (100 mg·kg <sup>-1</sup> )	Rice husk	500 °C, 3 h	5% by weight	AI, DI	Ammonia oxidizing bacteria	Enhancing ammonia oxidizing and N cycle	Li et al. (2021)
Cu (338 mg·kg <sup>-1</sup> )	Chicken manure	300 °C, 3.6 °C·min <sup>-1</sup> , 2 h	5% by weight	AI, DI	–	Enhancing C fixation	Moore et al. (2018)
Pb (496 mg·kg <sup>-1</sup> ) Cr (127 mg·kg <sup>-1</sup> ) As (701 mg·kg <sup>-1</sup> ) Cu (502 mg·kg <sup>-1</sup> ) Zn (274 mg·kg <sup>-1</sup> )	Pinecone	350 °C, 5 h	5% by weight	AI, DI	Bacillus, Lysinibacillus, Acinetobacter, Desulfotomaculum	Enhancing energy production and conversion in vivo, soil nutrient and detrimental substances transformation	Lan et al. (2021)
Cd (10 mg·kg <sup>-1</sup> ) Pb (150 mg·kg <sup>-1</sup> )	Sugarcane bagasse	400 °C	1% by weight	AI	–	Enhancing SOM decomposition, nutrient cycling, organic pollutants degradation	Azadi and Raiesi (2021)
Cu (338 mg·kg <sup>-1</sup> )	Chicken manure	500 °C, 2 h	3% by weight	AI, DI (especially towards fungi)	–	Enhancing substrate utilization and CO <sub>2</sub> emission	Meier et al. (2021)
Pb (230 mg·kg <sup>-1</sup> )	Wheat straw	350–500 °C	40t/ha	–	Ammonium oxidizing microbes	Enhancing soil denitrification and N <sub>2</sub> O emission	Zhou et al. (2018)
Cd (24 mg·kg <sup>-1</sup> ) Zn (399 mg·kg <sup>-1</sup> )	Cattle manure	500 °C	5% by weight	AI (with significant shift of community structure)	Gram-negative bacteria, Gram-positive bacteria	Higher enzyme activities, possibly accelerating soil nutrient cycle and enhancing soil fertility	Ducey et al. (2021)

**Table 2** (continued)

Contamination level	Biochar characteristics			Variation of soil conditions		Microbial response		References
	Feedstock	Pyrolysis treatment	Application rate	Abundance and diversity	Dominant species	Biological function		
Pb (81 mg·kg <sup>-1</sup> ) Cu (116 mg·kg <sup>-1</sup> ) Zn (341 mg·kg <sup>-1</sup> )	Rice husk	500 °C, 3 h	50% by weight	EC, available N increase and water-soluble organic carbon decrease	AI, DI Nitrobacterium, denitrifying bacterium	Higher activity of nitrite reductases (nirK), N <sub>2</sub> O emission reduction	Li et al. (2019)	
As (55 mg·kg <sup>-1</sup> ) Cd (0.46 mg·kg <sup>-1</sup> ) Cu (50 mg·kg <sup>-1</sup> ) Zn (101 mg·kg <sup>-1</sup> )	Rice straw, vegetable leaves, fresh soil, and bran	500 °C, 3 h	5% by weight	pH and water-soluble organic carbon, NO <sub>3</sub> -N increase	AD	Higher activity of increased ammonia monooxygenase and nitrification increase	Li et al. (2021)	
Cd (13 mg·kg <sup>-1</sup> )	Sugar-gumwood	550 °C	3% by weight	pH and DOC, basal and substrate-induced respiration increase	AI	CO <sub>2</sub> emission reduction	Bandara et al. (2021)	
Cu (278 mg·kg <sup>-1</sup> ) Pb (348 mg·kg <sup>-1</sup> )	Sugarcane bagasse	450 °C, 4 h	3 t/ha	Soil moisture content, pore volume, nutrient retention, SOM, CEC increase	AI, DI	Higher activity of urease, catalase and invertase	Nie et al. (2018)	
Mn, Cu, Zn	Wheat straw	400 °C	5 kg per plant	pH, SOM, CEC, available Ca, Mg, P increase	AI, DI	Promoting soil nutrient cycle and inhibiting plant pathogens	Zhang et al. (2021)	
As	Oil palm fibers	700 °C, 1.5 °C·min <sup>-1</sup> , 4 h	3% by weight	pH increases	ASL, DSL	Fe(III) reduction, As(V) desorption and reduction, As(III) release into soil	Qiao et al. (2018)	
Cd (43 mg·kg <sup>-1</sup> ) Pb (4687 mg·kg <sup>-1</sup> )	Nutshell	465 °C	5% by weight	pH, soil respiration, TOC increase	AI	Higher microbial carbon use efficiency and more CO <sub>2</sub> -C release	Xu et al. (2018)	
Pb (167 mg·kg <sup>-1</sup> ) Cd (25 mg·kg <sup>-1</sup> ) Cu (69 mg·kg <sup>-1</sup> ) Zn (225 mg·kg <sup>-1</sup> )	Rice straw	600 °C, 3 h	1% by weight	pH, OM increase	AI, DI	Higher activity of urease and alkaline phosphatase	Huang et al. (2017)	

**Table 2** (continued)

Contamination level	Biochar characteristics			Variation of soil conditions	Microbial response		References
	Feedstock	Pyrolysis treatment	Application rate		Abundance and diversity	Dominant species	
As (55 mg·kg <sup>-1</sup> ) Cd (0.46 mg·kg <sup>-1</sup> ) Cu (50 mg·kg <sup>-1</sup> ) Zn (101 mg·kg <sup>-1</sup> )	Rice straw	500 °C, 3 h	5% by weight	pH, OM, TOC, available P and K increase, EC slightly decrease	–	–	Higher activity of urease and inhibition of dehydrogenase, catalase, β-glucosidase, invertase Tang et al. (2020)
Cd (43 mg·kg <sup>-1</sup> ) Pb (4687 mg·kg <sup>-1</sup> )	Wheat straw	450 °C	40 t/ha	pH, SOC increase	–	–	Higher activity of activities of neutral phosphatase, urease, and sucrase Cui et al. (2013)
As (30 mg·kg <sup>-1</sup> )	Straw	600 °C, 10 °C·min <sup>-1</sup> , 1 h	1% by weight	pH, DOC, C/N ratio increase, DOC, TN, TC decrease	Al	–	– Zhang et al. (2020)



**Fig. 4** Triggers for negative/uncertain consequences after biochar application to soil. Negative effects are mainly ascribed to the release of detrimental substances and proliferation of pathogens. The uncertainty could be caused by the differences in microbial growth conditions, and hence a heterogeneous stimulation of various microbial species

specific species or increase the abundance of pathogens, leading to microbial abundance and species loss.

#### 4.2 Interspecific microbial relationships

Stimulation and activation effect of biochar can drive the substitution of dominant species and evolution of indigenous soil microbial community, which can influence intrinsic interspecific relationships of soil microbials. Besides, sorption of signal molecules and extracellular metabolites may disrupt interspecific behaviors.

Masiello et al. (2013) investigated the impact of biochar on interspecific communication of soil microbials. The inhibition on microbial gene expression due to the biochar-induced adsorption of N-3-oxo-dodecanoyl-L-homoserine lactone (intercellular signaling molecule of AHL expression) between sender and receiver cells was quantified via the difference in green fluorescence protein (GFP) expression level. In the biochar-mixed agar, GFP detected within the receiver cells was only  $24.1 \pm 2.1\%$  of control. Notably, the negative correlation between GFP expression level and content of surface functional groups suggested the inhibiting effect of biochar adsorption. Most recently, an electrochemical test observed that woody biochar can directly transfer electrons through carbon matrix in a relatively slow manner (e.g., charging and discharging circles of surface functional groups) (Sun et al. 2017). Therefore, the presence of additional electron acceptor or donor may disrupt interspecific metabolic cooperation (Masiello et al. 2013).

Since soil microorganisms live by interacting with each other (e.g., syntrophic co-metabolism, competition, production of inhibitors or activators, and predation), biochar-induced changes in microbial interspecific relationships could be basis in various microbial responses (Haruta et al. 2009). However, it remains unclear about the effect of biochar towards microbial communication (e.g., communication mode, microbial signaling, gene expression) on both short and long-time scale.

#### 4.3 Microbial community composition and structure

Microbial community is one of the most important indexes for soil microbiological health (e.g., structural and functional diversity), where the composition is subject to soil temperature, moisture, texture, nutrient availability, pH, and seasonality (Haruta et al. 2009). Microbial diversity indicates microbial resilience and adaptability towards environmental changes to some extent. The soil microbial community is highly sensitive, and from this perspective, biochar-induced microbial community evolution can be sorted as activation, expansion, substitution and adaption of several indigenous microbial species.

O'Neill et al. (2009) stated that k-strategists such as actinomycetes were presented as chief proportions of microbial community in biochar-rich soils. Similar result was observed in a biochar-amended paddy soil for the fact that actinomycetes and fungi increased by 38.5% and 930% owing to soil pH variation, respectively (Cui et al. 2013; Xu et al. 2021). Besides, microbial community composition in the “soil-PTE-biochar” system was synchronously subject to biochar properties, application rate and

mixing process. Ahmad et al. (2016) stated that feedstock of biochar plays a chief role in determining its outcomes of bacterial community and diversity, whereas pyrolysis temperature is considered as the second most important. Xu et al. (2017) revealed that wine-lees biochar improved microbial diversity while reduced the overall abundance in a multi metal-contaminated paddy soil, especially in the maturity stage.

Biochar-induced changes in the microbial community were uncertain, however, factors such as feedstock type, production parameters and application time can be decisive. The term “disturbance” referred to a biotic or abiotic cause which resulted in either a “perturbation” or a “stress”. Therefore, a series of changes induced by biochar application (discussed in 3.1 and 3.2) can result in changes of specific species, which triggered the subsequent microbial evolution and hence changes in ecological functions (discussed in 4.4). Moreover, according to the theory of “critical slowing down” (i.e., weaker resilience occurred at the tipping point between one stable state to another), the uncertainty in biochar-induced microbial community changes can be interpreted by the application time. For example, soil microorganisms may undergo the transitional stage in adaption of soils contaminated by PTEs. However, the survived or recovered species ultimately adapted to the new habitat with stable growth rates (Griffiths and Philippot 2013), which can be traced in genomic testing reports.

#### 4.4 Microbial ecological functions

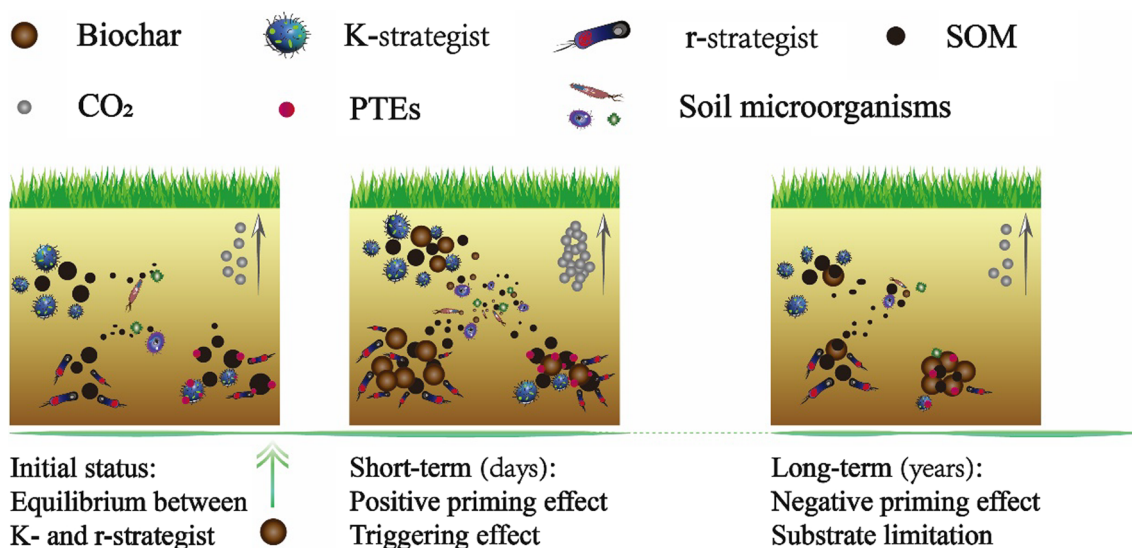
Enzymatic activity is considered as measurement of soil health (Acosta-Martinez et al. 2018), which is closely associated with microbial ecological functions. In general, biochar-mediated soil microbiological processes are mainly associated with soil nutrient turnover, agronomic values, as well as climate change mitigation (Tian et al. 2016).

Jain et al. (2016) found that biochar addition enhanced the resilience of soil enzymes associated with the nutrient cycle (e.g., acid/alkaline phosphatase, urease, arylsulphatase, dehydrogenase, phenol oxidases, cellulase and  $\beta$ -glucosidase), emphasizing the significant role of biochar-Fe complex due to its strong binding affinity. It was reported that 1% addition of wine-lees biochar dramatically stimulated the activity of urease in multi metal-contaminated paddy soil (Xu et al. 2017), which boosted the soil C cycle. Notably, moderate application of biochar may be cardinal to enzymatic activity maintenance due to their vulnerability and sensitivity (Jain et al. 2016; Tian et al. 2016). Mierzwa-Hersztek et al. (2016) proposed biochar-reinforced enzymatic activity may arise from the improvement of both substrates and moisture content, and besides, microbial activity and easily metabolizable

root exudates were also essential. Yet paradoxically, SOM stabilization was observed due to the limited microbial biomass and weak microbial metabolic quotient of soil microorganisms enclosed within biochar-induced aggregates (Wu et al. 2016).

With respect to agronomic benefits, biochar may enhance cohesion of soil particles and maintain the stability of soil aggregates and hence modify soil texture (Zheng et al. 2018). Furthermore, Matsubara et al. (2002) demonstrated when coconut biochar was incorporated in soil, both the incidence and severity of fusarium infection in asparagus plants were significantly reduced, which benefited AMF-asparagus symbiont colonization. Similarly, biochar-induced limitation on Fe accessibility, especially towards phytopathogenic fungi, contributed to corresponding disease control to a large degree (Gorovtsov et al. 2020). This effect would be further promoted when assisted with siderophores synthesis (Harindintwali et al. 2020). In PTE-contaminated fields, the presence of biochar presumably mediated expression of metal-immobilization related genes (Chen et al. 2018). Wang et al. (2019a, b) testified that microorganisms can alter surface structure of biochar, serving as the permeable coating which absorbed PTEs, where the PTEs pumped out from the cytoplasm can be feasibly immobilized by biochar.

PTE-induced inhibition of microbial metabolism may further consume SOM and result in greenhouse gas emission (discussed in 2.2.1). Therefore, the benefited soil microbiota may contribute to carbon sequestration. Firstly, biochar was widely acknowledged as a “carbon neutrality” approach. The condensed and amorphous polymeric carbon endow biochar with the recalcitrant nature, which favors soil carbon sequestration. Liu et al. (2020) verified the preferable carbon sequestration potential of biochar than traditional agronomic measures in upland soil and paddy soil (Liu et al. 2020). Moreover, biochar may affect native SOM mineralization rates through priming effects (Rasul et al. 2022). Masiello et al. (2013) insisted that biochar-resulted influence on soil carbon storage was subject to the sorption of cellular signals. Based on the origin of the released CO<sub>2</sub>, priming effects can be divided into apparent (e.g., originated from microbial compound turnover) and real (e.g., originated from SOM decomposition) types, and both positive and negative priming effects may occur in the two phases (Rasul et al. 2022). Biochar-induced positive priming effects can be translated as different responses, and hence equilibrium of both r-strategist (i.e., microbes that rapidly response towards carbon substrate and re-mineralize SOM through co-metabolism) and K-strategist microbes (i.e., microbes that continually consume SOM) (Aponte et al. 2020), ascribed to the release of liable OC. As for the negative priming effects, mechanisms such as



**Fig. 5** Priming effect of biochar in soil at various time scales. Biochar addition influences the initial equilibrium of K- and r-strategist microorganisms, and hence SOM mineralization. In the short period, abundance of soil microorganisms, especially r-strategist microorganisms may rapidly increase and boost SOM mineralization through co-metabolism. As for long term scale, due to the lack of available substrates, a negative priming effect can occur

the accumulation of recalcitrant carbon (Pei et al. 2020), improvement of organo-mineral interactions (Van Zwi-eten et al. 2017), and aggregate formation are involved. Moreover, the deleterious substances released from biochar may severely abolish microbial activities (Ghidotti et al. 2017). From the temporal perspective (Fig. 5), biochar addition may enhance SOC mineralization for a short period, while shows the negative priming effects due to the lack of SOC for long terms (Zhao et al. 2014). Besides, outcomes of priming effects are associated with incubation properties, soil conditions, biochar properties (e.g., C/O ratio, surface functional groups), and dominant microbial species (Budai et al. 2016).

However, most of the biological effects of biochar on soil carbon sequestration remain unknown. Current studies have focused more on the links between biochar-induced changes of microbial community and soil carbon dynamics (Bi et al. 2020). Many studies have verified the beneficial role of biochar-microbes interaction in soil carbon sequestration (Khadem et al. 2021). Biochar may enhance carbon utilization capacity of soil fungi that serve as a special substrate and thus promote community evolution, leading to increased carbon utilization efficiency along with attenuated SOM mineralization rates in upland soil and paddy soil, respectively. Besides, biochar can intensify nitrogen fixation ascribed to the release of B and Mo (Lehmann et al. 2011). Moogi et al. (2021) demonstrated that biological nitrogen fixation was increased by 1.44 times in biochar-modified soils with

30% atmosphere-derived N. They found that biochar has the potential to increase N input into agroecosystems as well as reduce NOx emissions. In a four-year field study, biochar addition decreased the ratios of abundance of methanogens to methanotrophs by 11–31% and thus decreased CH<sub>4</sub> emission by 20–51% annually (Wang et al. 2019a, b). However, the soil carbon sequestration potential may be reduced. In acid soils, biochar addition may increase the proportion of gram-positive bacteria that are responsible for the decomposition of biochar and SOC through co-metabolism and simultaneously lower the substrate limitation, causing more CO<sub>2</sub> release (Sheng et al. 2016). Overall, there is an urgent need for investigations on the potential mechanisms of biochar-microbes interaction to accurately evaluate the soil carbon sequestration potential of biochar.

### 5 Current challenges and perspectives

This paper reviewed the PTE-induced stress on soil microbials and the benefits of biochar application towards microbials. The complex mechanisms of interaction of biochar and soil microbials in presence of PTEs has been systematically summarized. Different microbial responses (e.g. positive, negative, and controversial) towards biochar application in PTE contaminated soil have been comprehensively discussed. Here, challenges and perspectives for future research development are also provided.



## 5.1 Challenges

Microcosm system, which is considered to be the closest system to a natural ecosystem, has been proposed to reflect the interactions between species and their habitats in recent years (Xu et al. 2021). It allows for deepening the mechanisms among interrelationships of “soil-biochar-PTE-soil microbiota” system, and hence links soil ecologic and social functions with microbial diversity and the expression of functional genes (Harter et al. 2014). Based on this, adopting the high-resolution technology such as PCR, DGGE, TGGE on the long-term in situ experiments may visualize overall microbial evolution by monitoring the changes in genera and even species levels. Regarding to changes in specific species or functional genes, cutting-edge analytical technologies such as the fluorescence in situ hybridization (FISH), DNA fingerprinting techniques and gene chips are urgently needed.

Except for state-of-the-art analytical technologies, advancing data analysis methods are also of pivotal importance. Traditional kinetic models can portray the dynamics of specific microbial indicators/characteristics over the experimental cycle. Nevertheless, it is a non-quantitative method due to the test intervals, which probably result in suspectable and ambiguous results. Mechanisms embedded in high-dimensional, redundant, and sequential in situ experimental data could be revealed using more appropriate approaches in the context of revising existing domain knowledge and theories. Advances in computational and system biology approaches such as metagenomics, metproteomics, and metabonomics provide comprehensive insights into the evaluation of microbial communities and biological functions. Recently, structural equation modeling analysis has exhibited superior performance for discriminating the effect of biochar properties and dose, soil properties on the microbial community (Xu et al. 2021). Besides, machine learning (ML) approaches can capture the complex nonlinear patterns in high-throughput data and hence extract the potential quantitative relationships between features (e.g., biochar-induced changes) and targets (e.g., microbial responses). However, the efficacy of ML approaches is highly dependent on the validity and volume of data, and trade-off between empirical utility and theoretical interpretability should also be taken into consideration.

Biochar application inevitably impacts soil microbials in PTE-contaminated soils, variably stimulates or inhibits microspecies, and thus drives the evolution of microbial community. Therefore, the degree and direction of microbial evolution could be determined from the equilibrium of microbial species in PTE-contaminated soils. The development of genomic techniques shifted the research hotspot on biochar-soil interactions into the

relationship between biochar-induced changes with species-level variations.

## 5.2 Perspectives

Understanding on factors that cause above species-level variations is still lacking, and hence more efforts should be made to deepen the understanding of biochar's effects on soil microbials in the presence of PTEs. For this purpose, three general issues in the perspective of fundamental research are needed to be given.

*Fit-for-purpose biochar* Only qualitative and semi-quantitative recognition between biochar properties and manufacturing parameters (e.g., feedstock type, pyrolysis temperature, residence time, heating rate) was established in existing research works (He et al. 2021). However, fit-for-purpose biochar production is critical for harnessing the commercial and environmental benefits of biochar. Advances in ML increased the possibility of accurate prediction of biochar properties, and minimized the experimental workload, demonstrating a bright future in the field. Relationships among biochar feedstock type and production conditions, biochar properties, biochar-induced changes in soils under different conditions and microbial composition, and microbial responses may be clarified and even regulated artificially. Undoubtedly, this is the foundation of the targeted application of biochar towards various agrotypes to manage soil microbiological health and promote sustainable agriculture and climate change.

*Total analysis and statistics* Current knowledge on the effect of biochar on soil microbials in PTE-contaminated soils is mostly empirical (Palansooriya et al. 2019). Given the intertwined nexus among soil parameters, we should strive to take various factors (e.g., constituent, and subsystem etc.) into consideration simultaneously. Based on this, we need to investigate the response of soil matrix (e.g., metagenomics, ionomics) during experiments at different scales. Besides, appropriate statistical methods should be introduced to explain the consequences in biochar-amended soils, and potential mechanisms. Furthermore, mechanisms concluded from total analysis and statistics may be combined with results from ML/DL models for further improvement of model performance and practical guidance.

*One health approach* The imperative goals of modern agriculture are to improve crop quality and reduce malnutrition. Albeit fertilization has supported the agronomic system worldwide, and appropriate alternatives are still required with respect to cost-effective and green chemistry in the future. Biochar is considered as a satisfying alternative for attaining environmental, ecological,

and economic sustainability (Jia et al. 2022). Therefore, governments should vigorously promote the sustainable land utilization awareness of farmers, as well as establish a sound system for biochar application through corresponding knowledge popularization, standards, preferential policies, and laws. For this goal, more efforts should be made with the cooperation among politicians, scientists, engineers, technicians, and agronomists.

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#### Author contributions

XY: Conceptualization, Funding acquisition, Writing—Review & Editing. MY: Writing—Original draft preparation, Literature search & Data analysis. SL: Writing—Review & Editing. BS: Review & Supervision. ZL: Writing—Review & Editing. XY: Conceptualization, Funding acquisition, Writing—Review & Editing.

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#### Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

#### Declarations

##### Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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