



Combined effects of earthworms and biochar on PAHs-contaminated soil remediation: A review

Shuyu Hou¹, Jue Wang^{2,*}, Jun Dai¹, Mohammed Boussafir³, Chi Zhang^{1,*}

¹ College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, China

² ISTO UMR 7327 CNRS-Université d'Orléans, Orléans, 45071, France

³ E.A 6293 GéoHydrosytèmes CONTinentaux (GeHCO), Faculté des Sciences et Techniques, Parc de Grandmont, 37200 Tours, France

* Corresponding authors. E-mail: jue.wang@univ-orleans.fr (J. Wang); zhangchi2012@scau.edu.cn (C. Zhang)

Received April 10, 2022; Revised August 29, 2022; Accepted August 31, 2022

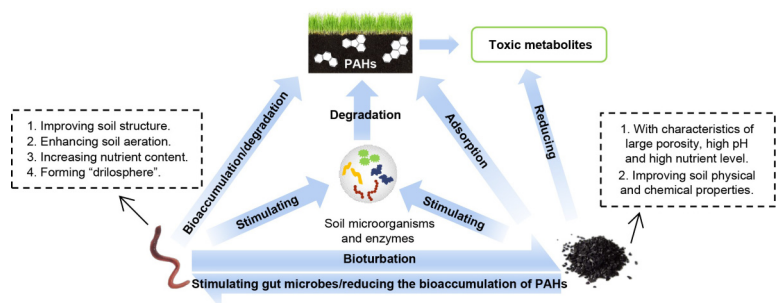
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ABSTRACT

- Earthworm remove PAHs from soil by bioaccumulation and stimulating microbial degradation.
- Biochar can adsorb PAHs and promote microbial degradation in soil.
- Earthworm improve the adsorption process of biochar by bioturbation.
- Biochar reduce the vermiaccumulation and improve the decomposition of PAHs by earthworm.

Polycyclic aromatic hydrocarbons (PAHs) in soil pose a threat to the health of humans and other organisms due to their persistence. The remediation method of combined application of biochar and earthworms has received growing attention owing to its effectiveness in PAHs removal. However, the earthworm–biochar interaction and its influence on PAHs in soil has not been systematically reviewed. This review focuses on the effectiveness of combined application of earthworms and biochar in the remediation of PAHs-contaminated soils and the underlying mechanisms, including adsorption, bioaccumulation, and biodegradation. Earthworm–biochar interaction activates the functional microorganisms in soil and the PAHs-degrading microorganisms in earthworm guts, promoting PAHs biodegradation. This review provides a theoretical support for the combined application of biochar and earthworms in the remediation of PAHs-contaminated soils, points out the limitations of this remediation method, and finally shows the prospects for future research.

Keywords earthworm, biochar, soil, PAHs, combined effect



1 Introduction

Soil can be both a source and sink for organic pollutants, including polycyclic aromatic hydrocarbons (PAHs) (Wild and Jones, 1995; Wilcke, 2000). PAHs are mainly produced by anthropogenic activities such as industrial production and daily life (Menzie et al., 1992; Edwards, 2004). They are hydrophobic, lipophilic, and of low volatility, and their hydrophobicity increases while volatility decreases with the increase in aromatic rings (Sims and Overcash, 1983). Some high molecular weight PAHs do not volatilize or photolyze under natural conditions. Therefore, they are persistent in soils (Sims and Overcash, 1983; Kuppusamy et al., 2017). Many PAHs are toxic, threatening human health after being amplified along the food chain (Diggs et al., 2011; Abdel-Shafy and Monsour, 2016). Sixteen

PAHs have been listed as priority pollutants by the US Environmental Protection Agency (Hartmann, 1996).

Traditional strategies use physical or chemical methods to remediate organic contaminated soils (Shi et al., 2020), including soil washing, steam extraction, electric remediation, oxidation, reduction, and dechlorination (Scullion, 2006). These methods are both costly and eco-unfriendly due to their complex processes and possibility of causing secondary pollution (Gan et al., 2009; Xu et al., 2018; Dhaliwal et al., 2020). Therefore, alternative techniques for organic contaminated soil remediation are urgently needed. Since the 1980s, eco-friendly remediation methods involving soil microbes, animals, and plants have been increasingly used (Vogel and Grbic-Galic, 1986; Wilson and Jones, 1993; Zou et al., 2000; Juwarkar et al., 2010; Ye et al., 2017; Rodriguez-Campos et al., 2019). Of these methods, vermiremediation has been shown to be an effective method for treating wastewater sludge and municipal solid wastes and removing organic contaminants in soils (Eijsackers et al.,

2001; Tharakan et al., 2006; Hickman and Reid, 2008; Rodriguez-Campos et al., 2014; Rorat et al., 2017; Zeb et al., 2020). In vermiremediation, earthworms change the physical and chemical properties of soils via burrowing and feeding, thereby increasing the availability of pollutants (Curry and Schmidt, 2007). Earthworms not only directly remove contaminants through absorbing and digesting but also indirectly remove contaminants by stimulating their degradation by microbes (Haimi, 2000; Sinha et al., 2010; Shi et al., 2014; Cao et al., 2015). The remediation method using biochar is also effective in organic pollutant removal from soils (Beesley et al., 2010; Chen et al., 2011a; Marchal et al., 2013). Biochar is a stable carbon-rich material produced through pyrolysis/carbonization of plant or animal biomass (Lehmann et al., 2011; Ahmad et al., 2014; Amoah-Antwi et al., 2020). Biochar application improves the soil biological environment and influences the physical and chemical transformation of soil pollutants (Lehmann et al., 2011; Chen and Yuan, 2011; Ahmad et al., 2014; Nsamba et al., 2015; Sadegh-Zadeh et al., 2017; Sandhu et al., 2017; Suliman et al., 2017; Wang et al., 2017a; Bielská et al., 2018; Toková et al., 2020; Siedt et al., 2021).

Although earthworms have been widely used to remove PAHs from soils, their ingestion and accumulation of contaminants can lead to the transfer of pollutants from soils to the food chain as many small mammals and birds prey on earthworms (Bergknut et al., 2007; Fagervold et al., 2010; Malev et al., 2016). In addition, the ingested PAHs can be excreted back to the soil by earthworms during their activities or released back to the soil when earthworms die and decompose (Coutiño-González et al., 2010). To find a more effective remediation method, many researchers have used earthworms and biochar together for pollutant removal and soil quality improvement (Gomez-Eyles et al., 2011; Wang et al., 2014; Zhang et al., 2019). Biochar has a high adsorption capacity for pollutants, thereby decreasing pollutant bioavailability in soil. It generally has a high pH and contains some toxic substances (Godlewska et al., 2021), which may have an adverse effect on earthworms. However, when applied in organic contaminated soils, it enhances pollutant degradation by microorganisms and reduces pollutant bioaccumulation by earthworms (Cao et al., 2011; Gomez-Eyles et al., 2011; Wang et al., 2012; Sanchez-Hernandez et al., 2019). Earthworms improve biochar migration in soil. Therefore, combined application of biochar and earthworms has been recognized as an effective and eco-friendly remediation method for organic contaminated soils (Eckmeier et al., 2007).

This review is based on a search of the literature published between 1970 and 2022 using six keywords: soil, earthworms, biochar, pollution, organic contaminants, and PAHs, and 319 articles were cited. The following three aspects are focused on in this review to provide an overview of recent developments in the combined application of earthworms and biochar in PAHs-contaminated soil remediation:

(1) The role earthworms play in the remediation of PAHs-

contaminated soils;

(2) Influencing factors of biochar characteristics during biochar production, and underlying mechanisms of PAHs-contaminated soil remediation using biochar;

(3) Earthworm–biochar interaction in PAHs-contaminated soil remediation and advantages and limitations of combined application of earthworms and biochar.

2 The role earthworms play in the remediation of PAHs-contaminated soils

Vermiremediation of organic contaminated soils is a complex process involving several mechanisms that are related to soil physical, chemical, and biological properties (Fig. 1) (Michael et al., 1997; Tiunov and Scheu, 1999; Contreras-Ramos et al., 2008; Qi and Chen, 2010). Vermiremediation has been proven to be effective in removing organic pollutants from soils, including PAHs, PCBs, and pesticides (Singer et al., 2001; Lin et al., 2012; Rodriguez-Campos et al., 2019).

2.1 The tolerance of earthworms to PAHs

To successfully apply vermiremediation in PAHs-contaminated soils, it is important to know the lethal dose values of PAHs for earthworms (Rodriguez-Campos et al., 2014). In the early 1980s, some researchers found that earthworms could survive and grow in soils with high concentrations of PAHs (Simmers et al., 1986; Hund and Traunspurger, 1994). Several studies have shown that earthworms could tolerate up to 1000 mg kg⁻¹ PAHs in soil or sediment with a survival rate over 80% (Eijsackers et al., 2001; Contreras-Ramos et al., 2006; Natal-da-Luz et al., 2012; Rodriguez-Campos et al., 2014). Gomez-Eyles et al. (2011) discovered that after 56 days of exposure to 773 mg kg⁻¹ PAHs in a soil, 97.5% earthworms survived. Even in soils with total petroleum hydrocarbon concentrations close to 12 000 mg kg⁻¹, earthworm densities as high as 512 individual m⁻² were found (Zavala-Cruz et al., 2014).

Although earthworms can tolerate high concentrations of PAHs, it is evident that their growth and reproduction are negatively affected by PAHs in soils and sediments (Eijsackers et al., 2001; Matscheko et al., 2002; Contreras-Ramos et al., 2006; Gomez-Eyles et al., 2011; Natal-da-Luz et al., 2012). The reduced reproductivity of earthworms in PAHs-contaminated soils could be a result of decreased protein and enzyme contents (e.g., catalase, cellulase, glutathione-S-transferase, and heat-shock proteins) (Tejada and Masciandaro, 2011; Xu et al., 2015). More detailed studies have shown that high levels of PAHs could damage the DNA in earthworms' seminal vesicles, resulting in a high degree of reproductive defect and a reduction in cocoon production (Eom et al., 2007; Tejada and Masciandaro, 2011; Gowri and Thangaraj, 2020; Li et al., 2020a; Zhang et al., 2020).

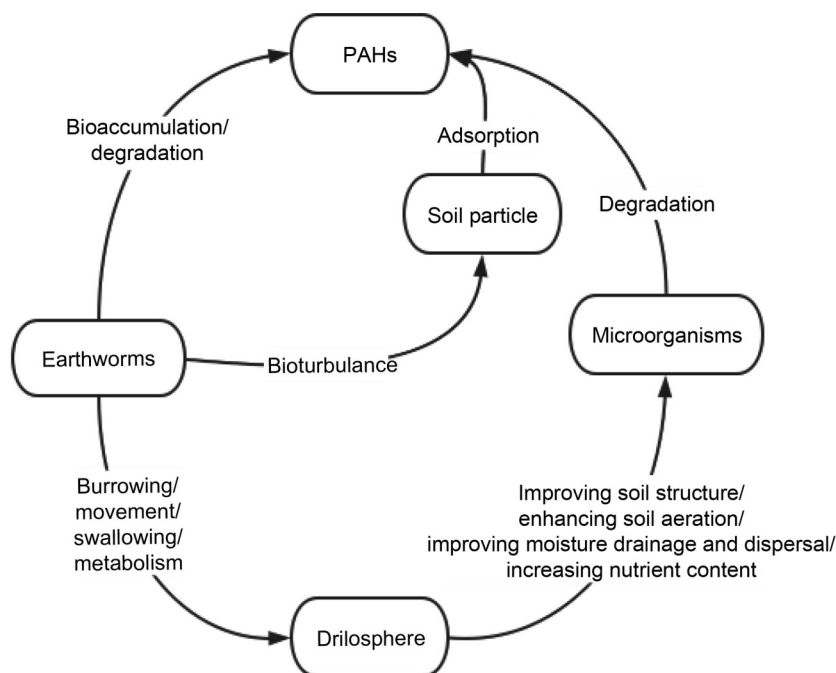


Fig. 1 Graphical representation of vermiremediation of PAHs-contaminated soil.

Application of organic materials to soil has been shown to mitigate the negative effects of PAHs on earthworms (Rodriguez-Campos et al., 2014). In a phenanthrene- and fluoranthene-contaminated soil, the weight loss of earthworms was 27% and 23.3% in treatments with low (10%) and high levels (40%) of organic amendment, respectively, and 25 more earthworm cocoons were produced in the high level treatment (Eijsackers et al., 2001). In the research of Contreras-Ramos et al. (2009), the weight of the earthworms (*Eisenia fetida*) increased by 35% when sewage sludge was added at 5% to the PAHs-contaminated soil, but decreased by 77% after 70 days due to a lack of food.

2.2 Vermiremediation mechanisms

2.2.1 Improvement of soil conditions by earthworm activities

It is well known that earthworm activities improve soil physical and chemical properties. Compacted clayey soils become loose and porous with the burrowing activity of earthworms, while loose sandy soils develop water-stable aggregates with the feeding and casting activities of earthworms (Barré et al., 2009). Therefore, earthworm activities improve the texture of loose or compacted soils, creating a better soil environment for soil microbes and plants (Barré et al., 2009). In addition, the earthworm bioturbation by earthworms enhances pollutant adsorption by soil particles (Hickman and Reid, 2008). In the drilosphere (i.e., burrow walls, gut contents, and casts) the activities of organic pollutant-degrading microorganisms are higher due to the good aeration, high moisture content, and rich nutrients (Michael et al., 1997; Tiunov and Scheu, 1999; Brown et al., 2000; Brown

and Doube, 2004). Mary et al. (2011) found that the total carbon content was 23% higher and the microbial biomass was 58% higher in the drilosphere than in the nearby bulk soil. It has also been proven that available nitrogen ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) is closely related to the metabolism of organic pollutants (Tran et al., 2013). The drilosphere indirectly promotes the degradation of organic pollutants by providing a favorable growth environment for the organic pollutant-degrading microbes (Tran et al., 2013).

2.2.2 Vermiaccumulation of pollutants

Vermiaccumulation is the process of contaminant absorption and accumulation by earthworms (Shi et al., 2020). There are two ways of vermiaccumulation of PAHs, passive epidermal uptake and dietary uptake (Shi et al., 2014). The first is related to PAHs with low hydrophobicity, including some volatile PAHs in soil air, while the second is related to highly hydrophobic PAHs (Lu et al., 2004; Qi and Chen, 2010). In other words, highly hydrophobic PAHs accumulate in earthworm guts, while PAHs with low hydrophobicity not only accumulate in earthworm body walls but also in earthworm body fluid and guts as a result of passive diffusion and partition (Shi et al., 2014). Earthworm gut fluid can markedly enhance the release of PAHs from soil particles (Qi and Chen, 2010). The high specific surface area of earthworm gut walls (Qi and Chen, 2010) and the strong diffusion of PAHs in gut fluid (Mayer et al., 2007) reasonably explain the high absorption of PAHs by earthworms. In soils contaminated with petroleum hydrocarbons (kerosene, diesel, and bunker-C), earthworms (*Eisenia fetida*) were found to accumulate the 16 priority PAHs (Moon et al., 2013). In ecological risk evaluation, the biota-sediment accumulation factor

(BSAF) is usually used to estimate contaminant loads in biota. In their study with a soil contaminated with PAHs (0.25–25 mg kg_{dwt}⁻¹), Jager et al. (2003) found a mean BSAF value of 0.23 (kg_{OC} kg_{lip}⁻¹) for the PAHs in the earthworms *Eisenia andrei*. Cachada et al. (2018) reported BSAF values of 0.41–2.5 and 0.026–0.16 (kg_{OC} kg_{lip}⁻¹) for PAHs in artificially and naturally contaminated soils, respectively. Compared with other PAHs, 4-ring PAHs display a higher accumulation in earthworms, which may be due to the fact that they do not volatilize or degrade, and they are not as persistent as 5–6-ring PAHs (Northcott and Jones, 2001).

2.2.3 Microbial and enzymatic PAHs degradation enhanced by earthworms

Earthworm bioturbation can effectively enhance PAH biodegradation by soil microorganisms which have limited access to PAHs due to their poor mobility (Dendooven et al., 2011). As mentioned above, earthworm bioturbation improves soil aeration and nutrient accessibility, which is favorable for aerobic PAH-degrading microbes (Binet et al., 1998; Marinari et al., 2000; Hickman and Reid, 2008; Pagenkemper et al., 2015). It is worth noting that earthworm gut is a favorable environment for anaerobic PAHs-degrading microorganisms due to the rich organic substrates (Drake and Horn, 2007), and efficient degradation of PAHs and other organic pollutants by earthworm intestinal bacteria has been reported (Contreras-Ramos et al., 2008; Verma et al., 2011).

Produced by plants, animals, and microorganisms, soil enzymes play an important role in the degradation of organic pollutants (Alkorta et al., 2003). Generally, soil enzyme activity is highly correlated with soil organic carbon content and microbial biomass (Martens et al., 1992; Fraser et al., 1994; Parthasarathi and Ranganathan, 2000). Studies showed that earthworms could increase the activities of organic pollutant-degrading enzymes, such as phosphatase, cellulase, amylase, sucrase, urease, catalase, and dehydrogenase (Zhang et al., 2000; Alkorta et al., 2003; Adetunji et al., 2017). In addition, the enzymes in earthworms generally have a high tolerance against organic contaminants, which is of significance for the biodegradation of organic pollutants (Schreck et al., 2008; Liu et al., 2011; Wu et al., 2011).

2.3 Vermiremediation of PAHs-contaminated soils

Many studies have shown that adding earthworms to PAHs-contaminated soils or PAHs-containing sewage sludge could accelerate the removal of PAHs while the earthworms maintain a high survival rate (Contreras-Ramos et al., 2008; Poluszyńska et al., 2017; Rorat et al., 2017). Ma et al. (1995) demonstrated that the degradation of anthracene and phenanthrene in 650 g soil was enhanced by the presence of five earthworms (*Lumbricus rubellus*), and the removal

rate of phenanthrene was increased from 11% to 25%. In the study of Contreras-Ramos et al. (2008), more than 90% of the anthracene (1000 mg kg⁻¹) and phenanthrene (150 mg kg⁻¹) and 16% of the benzo(a)pyrene (150 mg kg⁻¹) were removed from the 50 g soil 11 weeks after 10 earthworms (*Eisenia fetida*) were added, and more than 80% of the earthworms survived. According to Coutiño-González and his colleagues (2010), earthworms (*Eisenia fetida*) not only enhanced the degradation of anthracene but also enhanced that of 9,10-anthraquinone, the most abundant degradation product of anthracene.

Earthworms application can effectively improve microbial degradation activity (Sun et al., 2011; Hernández-Castellanos et al., 2013). Sun et al. (2011) reported 1.2 to 1.6 times increases in the microbial degradation rate of pyrene with the addition of earthworms (*Eisenia fetida*) to the artificially contaminated soils. Hernández-Castellano et al. (2013) observed that the addition of earthworms (*Pontoscolex corethrurus*) accelerated the removal of benzo(a)pyrene by four times. They also demonstrated that feeding the earthworms with *M. pruriens* or *B. humificola* leaves further increased the benzopyrene removal rate by 28.6% and 34.2%, respectively. Vermiremediation has been extensively applied in various soils (Parrish et al., 2006; Rodríguez-Campos et al., 2014; Rorat et al., 2017). It is worth noting that the growth and activity of earthworms are influenced by many factors, such as environmental pH, temperature, humidity, food, and toxic substances (Eom et al., 2007; Eijsackers et al., 2001; Owojori and Reinecke, 2010; Dendooven et al., 2011; Tejada and Masciandaro, 2011; Shi et al., 2020). Therefore, the effectiveness of vermiremediation is influenced by many factors, which warrants more studies. In addition, the fate of the PAHs accumulated by earthworms and the fate of toxic degradation by-products warrants further investigation as well (Coutiño-González et al., 2010; Schmidt et al., 2017).

3 The application of biochar in the remediation of PAHs-contaminated soils

Having a porous structure and a large specific surface area, biochar is increasingly used as an adsorbent in soil remediation (Alexander, 2000; Cornelissen et al., 2005; Zhu et al., 2017; Wang et al., 2020). In addition, biochar is carbon-rich and thereby a good habitat for soil microbes, which indirectly promotes the transformation and degradation of contaminants in soil (Fig. 2).

3.1 Influencing factors of biochar production and biochar characteristics

Feedstock for biochar production can be industrial and agricultural wastes, such as animal wastes, wood chips, crop residues, ash from power stations, and sewage sludge from wastewater treatment plants (Chen et al., 2011a; Cantrell

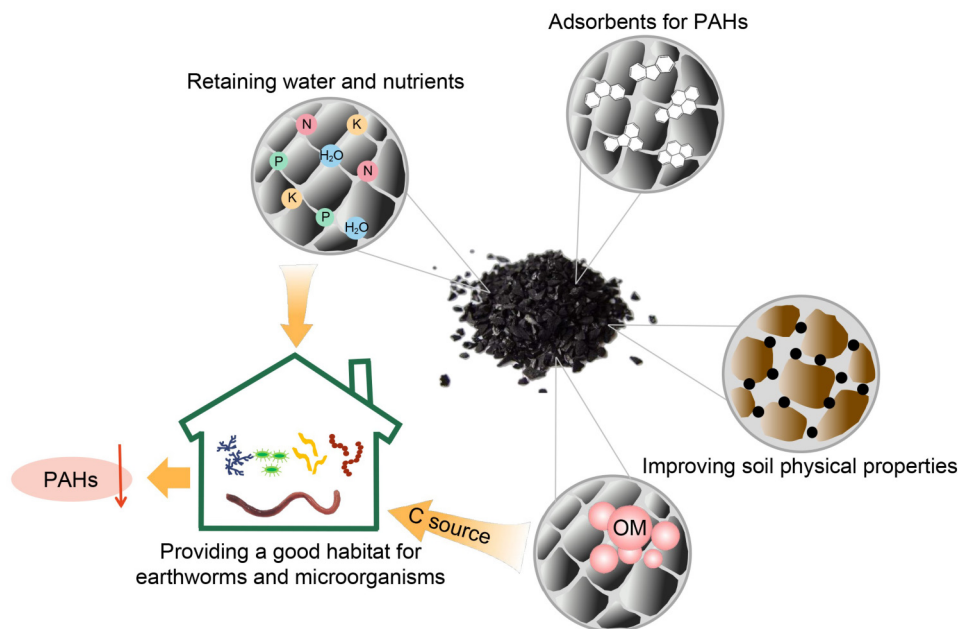


Fig. 2 The role of biochar in the remediation of PAHs-contaminated soil.

et al., 2012; Beesley et al., 2014; Rey-Salgueiro et al., 2016; Peng et al., 2018). Feedstock greatly influences biochar characteristics, including porosity, specific surface area, pH, electrical conductivity, cation exchange capacity (CEC), and elemental content (Gonzaga et al., 2017; Li et al., 2017a; Sandhu et al., 2017; Sun et al., 2018). In the study of Bianco et al. (2021), the total carbon content of biochar derived from different feedstocks varied widely from 22% to 83%. In the study of Gomez-Eyles et al. (2013), acai pit biochar had the highest carbon content and the largest specific surface area, while pine dust biochar had the lowest, with those of peanut hull biochar and barley straw biochar in between.

Based on production conditions, there are four biochar production technologies: slow pyrolysis, fast pyrolysis, gasification, and torrefaction (Wang et al., 2020). Biochar characteristics, such as porosity, surface area, carbon content, and stability, are greatly influenced by production conditions, such as pyrolysis temperature, heating rate, and pyrolysis atmosphere (Gonzaga et al., 2017; Kalinke et al., 2017; Sun et al., 2018; Wang et al., 2020a, 2021). Generally, biochar with a higher carbon content and a larger specific surface area can be obtained at a higher pyrolysis temperature (Chen et al., 2008; Li et al., 2013). This can be due to the release of volatiles and the generation of vascular bundle structures at high pyrolysis temperatures, which lead to a larger specific surface area and a pore structure of biochar (Fu et al., 2011; Li et al., 2013; Wang et al., 2020a). However, too high a pyrolysis temperature would destroy both the acidic and basic groups, leading to an ultimate decrease of the adsorbability of biochar (Chun et al., 2004; James et al., 2005). Slow heating rate and long residence time in slow pyrolysis achieve a high production yield of biochar, while the fast heating rate ($10\text{--}200\text{ K s}^{-1}$) and short residence time ($0.5\text{--}10\text{ s}$) in fast pyrolysis favors the

production of bio-oil (Perego and Bosetti, 2011; Oni et al., 2019; Lee et al., 2020). Long vapor residence time allows the biomass components to repolymerize, increasing the porosity and production yield of biochar (Tripathi et al., 2016; Tsai et al., 1997).

3.2 Remediation mechanisms of biochar

3.2.1 Physicochemical adsorption

The physical and chemical properties of biochar are the main factors affecting its adsorption of organic pollutants (Zhu and Pignatello, 2005). The interaction between biochar and organic pollutants is also influenced by the structure and chemical properties of the organic pollutants (Inyang and Dickenson, 2015). The interactions between biochar and PAHs (Fig. 3) mainly include hydrophobic interaction (e.g., Van der Waals forces), donor-acceptor interaction (e.g., $\pi\text{-}\pi$), and other specific interactions (e.g., hydrogen- π , cation- π) (Anyika et al., 2014; Fu et al., 2018; Zhang et al., 2018b; Bianco et al., 2021). The adsorption dominated by hydrophobic interaction can occur in two main phases. One is the partition of PAHs in the hydrophobic domain of biochar, and the other is the weak adsorption of PAHs on the surface of biochar via van der Waals force (Bianco et al., 2021). It has been revealed that the PAHs-adsorption mechanisms differ for biochar prepared at different pyrolysis temperatures. The adsorption of PAHs on low-temperature pyrolyzed biochar is primarily determined by the distribution of non-carbonized biopolymers and van der Waals force (Chen et al., 2012b; Zhu et al., 2018). High-temperature pyrolyzed biochar shows a greater sorption efficiency due to a lower H/C ratio and more unsaturated functional groups,

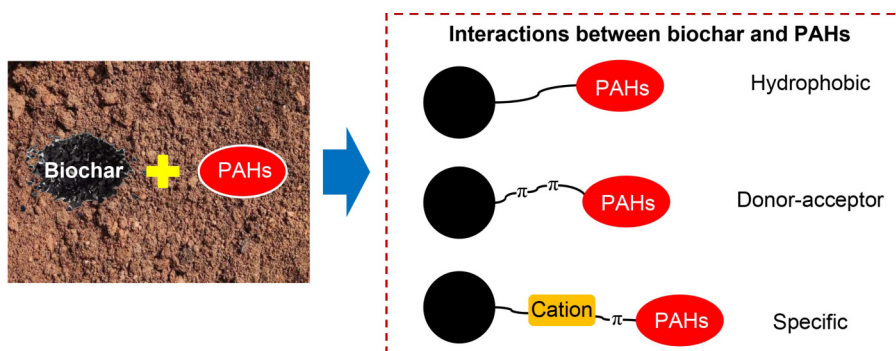


Fig. 3 Interactions between biochar and PAHs.

and it can interact with PAHs through π - π type interaction (Chen et al., 2008; de Jesus et al., 2017). Certain cations (e.g., Na^+ and K^+) and functional groups (e.g., $-\text{COOH}$, $-\text{OH}$, and $-\text{NH}_2$) in biochar undergo specific interactions with PAHs (Yang et al., 2016). The hydrophobic functional groups on biochar surface create a hydrophobic microenvironment, promoting the formation of cationic π -complexes, increasing the aqueous solubility of PAHs (Chen et al., 2007; Jin et al., 2014), and ultimately reducing the adsorption of PAHs by biochar. However, the formation of cation-PAHs bond increases the adsorption of PAHs on biochar. Therefore, PAHs adsorption or dissolution is a result of the relative strength of the PAHs-biochar interactions (Zhang et al., 2011; Jin et al., 2014).

3.2.2 Improving the soil environment by biochar

Biochar application alters soil physical and chemical properties, such as pH, porosity, and water holding capacity, creating hotspots of microbial growth. When applied to acidic soils, alkaline biochar improved the habitats of soil microorganisms (Birk et al., 2008; Van Zwieten et al., 2010). Moreover, owing to its porous structure, high porosity, and large specific surface area, biochar increases soil porosity, CEC, and water holding capacity when applied to soil (Glaser et al., 2002; Karhu et al., 2011; Ameloot et al., 2013; Zhu et al., 2017). Biochar also enhances microbial degradation of organic pollutants by supplying simple organic substrates like sugars and amino acids (Cox et al., 2001; Sadegh-Zadeh et al., 2017). In addition, the black color of biochar helps to raise soil temperature by efficiently absorbing solar radiation, speeding up organic matter decomposition and nutrient transformation (Maroušek et al., 2018; Amoah-Antwi et al., 2020).

3.3 Positive roles of biochar in PAHs-contaminated soil remediation

3.3.1 Adsorption removal of PAHs from soils using biochar

The strong adsorption capacity of biochar leads to the immobilization of PAHs and thus reduces the extractability

and bioavailability of PAHs in the environment, thereby reducing the potential health risk of PAHs (Alexander, 2000; Cornelissen et al., 2005; de Resende et al., 2018). Biochar can decrease the bioavailability of PAHs and thereby has been widely applied to PAHs-contaminated soils and sediments (Cornelissen et al., 2005; Beesley et al., 2010; Yang et al., 2018). Zhu et al. (2018) demonstrated that biochar reduced the concentrations of extractable PAHs. In the field experiment conducted by Beesley et al. (2010), the concentrations of the heavier, more toxic 4- and 5-ring PAHs decreased by more than 50% and those of the lighter 2- and 3-ring PAHs decreased by more than 40% 60 days after biochar application. Khan et al. (2013) added sewage sludge biochar (10%) to a PAHs-contaminated soil and found that the total concentration of PAHs in soil decreased by 10%, and the average concentration of PAHs in lettuce plants decreased from 1.72 mg kg^{-1} to 0.68 mg kg^{-1} . It is worth mentioning that soil-PAHs interaction is generally weaker than biochar-PAHs interaction. Therefore, soil-bound PAHs are at a higher risk of being redistributed into soil solution (Zhu et al., 2018). Biochar application increases the adsorption of pollutants to the soil matrix and thereby reduces their presence in soil solution. The adsorption-desorption experiment conducted by Zhang et al. (2010) showed that biochar addition enhanced the apparent adsorption affinity of soil to phenanthrene, which became more obvious at higher application rates, and biochar pyrolyzed at a higher temperature was more able to promote the adsorption of phenanthrene in soil.

3.3.2 PAHs biodegradation enhanced by biochar

Biochar cannot only effectively reduce the extractable and bioavailable PAHs concentration in soil by adsorption but also promote the biodegradation of PAHs by stimulating microorganisms, thereby reducing the environmental risks of PAHs (Beesley et al., 2010; Zhang et al., 2018a). This seems to contradict the fact that reduced extractability and bioavailability would lead to reduced biodegradation efficiency, and consequently, more pollutant would remain in the soil (Arp et al., 2014; Xiong et al., 2017). For example, the addition of black carbon significantly reduced the extractability and microbial mineralization of hydroxypropyl-

cyclodextrin and phenanthrene in soil (Rhodes et al., 2008; Rhodes et al., 2010). Some studies argue that biochar, with a high porosity, a large specific surface area, and rich nutrients, provides a suitable shelter for microorganisms and increases the abundances of pollutant-degrading bacteria (Anyika et al., 2014; Kong et al., 2018; Ye et al., 2019). It is generally believed that contaminant-degrading bacteria degrade the organic contaminants dissolved in soil solution or desorbed from adsorption sites. However, biodegradation of contaminants adsorbed on biochar has also been observed by some researchers (Park et al., 2003; Cornelissen et al., 2005; Chi and Liu, 2016; Ding et al., 2021). For example, Ding et al. (2021) observed that there were pollutant-degrading bacterial assemblages on the biochar retrieved from the contaminated soil in their experiment, indicating the possibility that contaminants adsorbed by biochar undergo biodegradation as well. Moreover, several studies have shown that the degradation of PAHs is affected by molecular weight. Biochar addition reduces the biodegradation of light PAHs while increasing that of heavy PAHs. This is because light PAHs are small and thereby can be easily adsorbed in the pores of biochar (Wang et al., 2006; García-Delgado et al., 2015; Zhang et al., 2018a).

In conclusion, the use of biochar is a reliable and sustainable method for soil organic pollution remediation. PAHs in contaminated soils can attach to the surface of biochar via specific adsorption, resulting in significantly lower concentration in soil solution. Biochar can also alter soil characteristics,

improving the soil environment for more efficiently PAHs removal by functional microbes. According to previous studies, the degradation rate of PAHs in soil increases with biochar application time (Table 1). However, much work is still needed to make it clear how to efficiently remove PAHs from soil, because removal efficiency varies with the application rate of biochar produced under different pyrolysis conditions and from different raw materials (Table 1). It is worth mentioning that biochar may contain some toxic by-products, which may have negative impacts on soil organisms. In some cases, the high pH and salinity of biochar may cause some undesirable results, and its high application rate is a disadvantage as well. Fortunately, these can be avoided by choosing a desirable feedstock and the optimal pyrolysis conditions (Lehmann et al., 2011; Quilliam et al., 2013; Luo et al., 2014; Godlewska et al., 2021).

4 Combined effects of biochar and earthworms on PAHs-contaminated soil remediation

Both earthworms and biochar can be used for soil remediation. However, both have their own shortcomings when they are used alone. Recently, some researchers have applied biochar and earthworms together in organic polluted soils to optimize remediation methods (Table 2). As reviewed in the previous sections, the mechanisms of PAHs removal by

Table 1 The effects of biochar type, application rate, and application time on the removal efficiency of $\Sigma 16$ PAHs

Feedstock	Pyrolytic temperature (°C)	Addition ratio	Time (d)	$\Sigma 16$ PAHs removal efficiency	Reference
Agricultural wastes					
Walnut shells, corn cobs	250, 400, 600	2%	75	73.69%–77.01%	Zhang et al., 2021
Rice husk	350	4%	90	7.69%–36.23% (The removal efficiencies of different PAHs)	Liu et al., 2015
	500			–32.67%–86.67% (The removal efficiencies of different PAHs)	
Dairy manure	350			–8.42%–71.74% (The removal efficiencies of different PAHs)	
	500			–4.21%–66.67% (The removal efficiencies of different PAHs)	
Wheat straw	700	5%	30	15.85%	Oleszczuk et al., 2017
Wood materials					
Poplar woodchips	500	1%	105	20.53%	Ren et al., 2021
Various types of conifers	>630	5%	270	41%	Ukalska-Jaruga et al., 2019
Hardwood	600	10%	56	31.84%	Gomez-Eyles et al., 2011
Hardwood	–	30%	60	About 50%	Beesley et al., 2010
Solid wastes					
Sewage sludge	500	1%	180	18%–56%	Tomczyk et al., 2020
Sewage sludge	500, 600, 700	2%	180	27%–4%	Godlewska et al., 2022
Sewage sludge	500	10%	56	10%	Khan et al., 2013

Table 2 The effects of carbonaceous materials on earthworms in organic contaminated soil and the bioaccumulation of organic pollutants in the presence of earthworms and carbonaceous materials

Pollutants	Carbonaceous materials	Application rate	Species	Population of earthworms	Duration	Biochar-amended soil	Weight	Survival rate	Bioaccumulation	Reference
PAHs	Hardwood-derived biochar	-	<i>Eisenia fetida</i>	10	28 d	200 g	Decreased by 2.9%	100%	Decreased by 22%	Gomez-Eyles et al., 2011
PAHs	Powder activated carbon	2% of soil weight	<i>Eisenia fetida</i>	30 (field experiment), 10 (laboratory)	56 d	5000 g (field experiment), 500 g (laboratory)	Decreased by 4.9%	100%	Decreased by 44.9%	Jakob et al., 2012
PAHs	Granular activated carbon						Decreased by 32% (laboratory)		Decreased by 47%	
PAHs	Wine tree cuttings biochar	0, 8, 16, 64, 128, 256, 1024 t ha ⁻¹	<i>Eisenia fetida</i> , <i>Eisenia andrei</i>	10	60 d	800 g	Decreased by 8% (laboratory)	100%, 100%, 100%, < 70%, < 60%, 0, 0	Increased by 54%	Malev et al., 2016
PAHs	Hardwood lump Charcoal biochar						Decreased by 43% (field experiment)		Increased by 10.7%	
PAHs	Wine tree cuttings biochar	100 t ha ⁻¹		30	48 d	2000 g	Decreased by 8%	80.8%	Increased by 54%	
PAHs	Hardwood lump Charcoal biochar						Decreased by 8%	60.9%	Increased by 10.7%	
PCBs	Wood waste biochar	0.7% of soil weight	<i>Eisenia fetida</i>	50	50 d	2250 g	-	30.8%	-	Denyes et al., 2012
PCBs	Rice husk biochar	2.8% of soil weight						70%	Decreased by 53%	
PCBs		11.1% of soil weight						35.2%	Decreased by 88%	
PCB28		1%	<i>Eisenia fetida</i>	25	24 d	1000 g	-	-	Decreased by 91%	Silvani et al., 2019
PCB101		4%						-	Decreased by 77%	
PCB28									Decreased by 87%	
PCB101									Decreased by 69%	
PCB28	Mixed wood shavings biochar	1%							Decreased by 55%	
PCB101									Decreased by 43%	

(Continued)

Pollutants	Carbonaceous materials	Application rate	Species	Population of earthworms	Duration	Biochar-amended soil	Weight	Survival rate	Bioaccumulation	Reference
PCB28	5%	Decreased by 63%								
PCB101		Decreased by 31%								
DDT	Activated carbon	0.2%	<i>Eisenia fetida</i>	5	28 d	200 g	-	-	Decreased by 83.9%–99.4%	Wang et al., 2018
	Biochar	2%								
		0.2%								
		2%								
Atrazine	Dairy manure biochar	0	<i>Eisenia fetida</i>	12	210 d	500 g	-	-	Decreased by 47%–73%	Cao et al., 2011
		2.5%					Increased by 19%	100%		
		5%					Increased by 26%	100%		
Pesticide mesotrione	Wheat straw-derived biochar	1%	<i>Eisenia fetida</i>	10	28 d	500 g	-	100%	Decreased by 80%	Zhang et al., 2019
		3%						100%	Not detected	
		10%						100%	Not detected	

earthworms and biochar include biochar adsorption, earthworm accumulation, enhanced biodegradation by soil microorganisms and enzymes, and metabolic degradation by the bacteria in earthworm gut. Biochar and earthworms are inevitably affected by each other in soil (Fig. 4). In this section, the potential of combined usage of biochar and earthworms in PAHs removal and the possible negative effects are discussed.

4.1 The effects of biochar on vermiremediation of PAHs-contaminated soils

4.1.1 Biochar addition improves the soil environment for vermiremediation

Owing to the porous structure of biochar (Downie et al., 2009), its tensile strength permits changes in soil mechanical impedance (Goss, 1977; Goss and Russell, 1980). Therefore, biochar application improves the soil environment for easier elongation and proliferation of plant roots (Bengough and Mullins, 1990; Blanco-Canqui, 2017). In addition, biochar application improves soil water and nutrient conditions, leading to better growth and development of plants, soil microorganisms, and animals (Warnock et al., 2007; Major et al., 2010; Noguera et al., 2010; Ren et al., 2018; Palansooriya et al., 2019). Biochar generally has a high pH

due to the presence of such salts as calcite (CaCO_3) (Cao et al., 2011). Therefore, biochar application is beneficial for earthworms in acidic soils with its acid-neutralizing effect, because earthworms prefer a neutral environment (Edwards and Bohlen, 1996; Tripathi and Bhardwaj, 2004; Van Zwieten et al., 2010). It is important to note that biochar could have the opposite effect when applied to neutral or alkaline soils (Van Zwieten et al., 2010), especially when it is overused (Weyers and Spokas, 2011). Biochar, with a large specific surface area and a porous structure, functions similarly to soil aggregates in terms of retaining water and nutrients and providing a good habitat for soil organisms (Tisdall and Oades, 1982; Lehmann et al., 2011). Compared to many organic substances, biochar can remain stable in soil for a long time (Skjemstad et al., 1996; Lehmann et al., 2005, 2008), even though it may become smaller on a decadal time scale (Nguyen et al., 2008). However, some researchers have pointed out that the strong adsorbability of biochar may reduce the availability of food and water for earthworms in soil (Jonker et al., 2004; Jakob et al., 2012; Zhang et al., 2019). Earthworms usually ingest both carbon and soil particles instead of soil particles only, which may be due to the detoxification and alkalisation effects of biochar, as well as its stimulating effect on microbial communities (Topoliantz and Ponge, 2003). Therefore, overuse of carbon may lead to less food available to earthworms (Wang et al., 2012), eventually leading to weight loss of earthworms

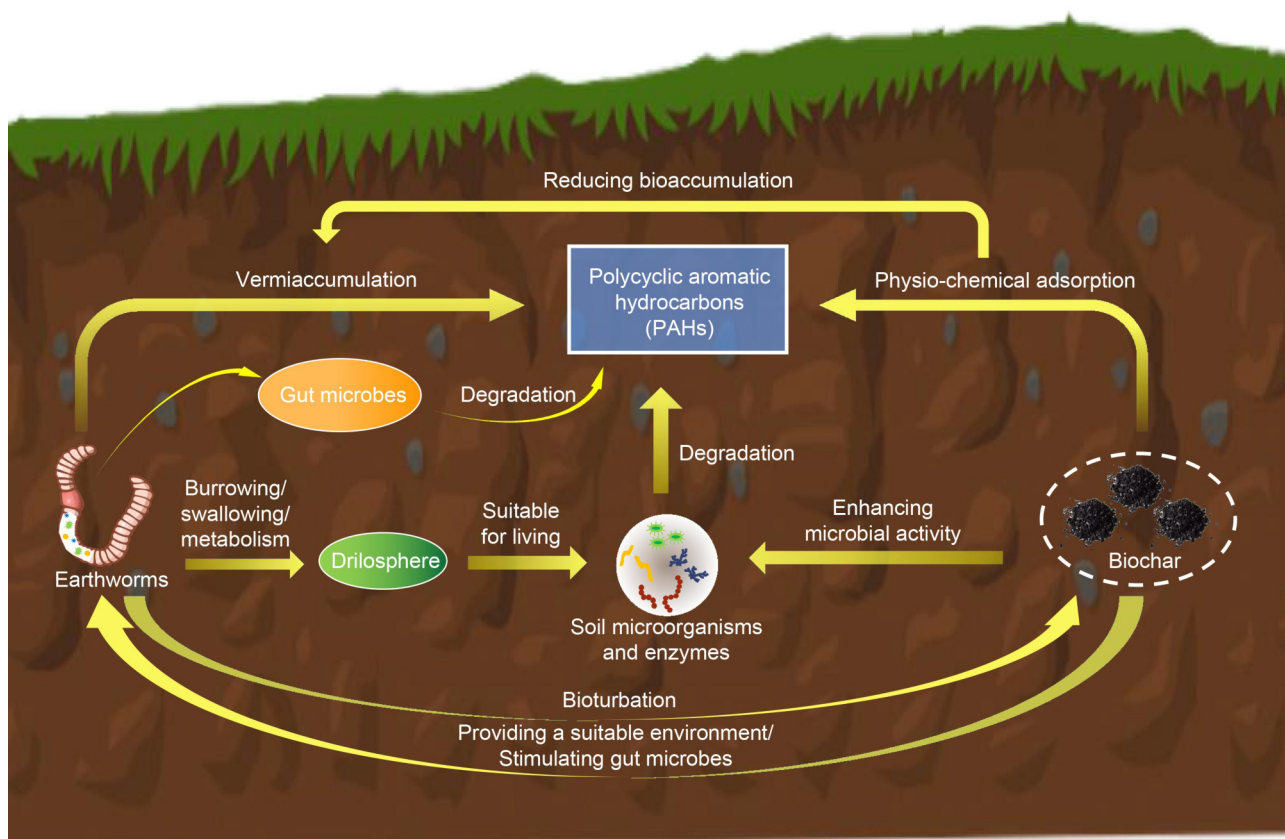


Fig. 4 Graphical representation of how earthworm and biochar work.

(Gomez-Eyles et al., 2011; Zhang, et al., 2019). In addition, earthworms may be subjected to drought stress caused by the application of biochar, which has a strong water-adsorption capacity (Li et al., 2011). To avoid this, irrigation may be needed immediately after biochar application (Domínguez and Edwards, 1997). For example, Li et al. (2011) observed the avoidance behavior and weight loss of earthworms in soils amended with 100 and 200 mg kg⁻¹ biochar, and no avoidance behavior was observed after the soil was irrigated to field capacity.

4.1.2 Biochar affects PAHs accumulation and degradation in earthworms

The hypoxic, humid, and pH-neutral environment of earthworm gut makes it an ideal habitat for pollutant-degrading microorganisms (Drake and Hrn, 2007; Van Groenigen et al., 2019). Microbes in earthworm gut have been proven to be able to effectively degrade PAHs. These microbes include bacteria (e.g., *Pseudomonas* and *Acidobacterium*) and fungi (e.g., *Penicillium*, *Mucor*, and *Aspergillus*) (Pižl and Nováková, 2003; Singleton et al., 2003; Contreras-Ramos et al., 2008). As mentioned above, earthworms prefer to feed on both soil particles and biochar rather than soil particles only (Topoliantz and Ponge, 2003). The surface of biochar is rich in water-soluble organic substances such as alcohols, aldehydes, ketones, and sugars, which are good substrates for the intestinal bacteria in earthworms (Thies and Rillig, 2009). Ding et al. (2019) reported that 0.5% rice biochar significantly increased the gut bacterial community in earthworms but had no effect on the soil bacterial community. Wang et al. (2020) also showed that biochar application significantly increased the bacterial diversity in earthworm gut, which was favorable for pollutant biodegradation.

The strong adsorption of pollutants on biochar not only reduces pollutant concentration in soil solution (Wang et al., 2012), but also effectively reduces pollutant accumulation by soil organisms (Alexander, 2000; Cornelissen et al., 2005; Langlois et al., 2011; de Resende et al., 2018), thereby lowering the risk of organic pollutants entering the food chain. This explains why earthworms prefer biochar-treated soil where the concentration of bioavailable contaminant is lower (Gomez-Eyles et al., 2011; Wang et al., 2014). Besides biochar, some other carbonaceous materials, such as black carbon and activated carbon, can also effectively reduce the accumulation of PAHs by earthworms (Brändli et al., 2008; Jakob et al., 2012). However, the reduction of bioavailable pollutants by biochar is influenced by soil properties. Earthworms absorb lower amounts of PAHs from clay loam than from sandy soil because the interaction between PAHs and clay loam is stronger than that between PAHs and sandy soil (Malev et al., 2016). Wang et al. (2012) pointed out that soils of different properties have different adsorption capacities for pollutants. Therefore, the effectiveness of biochar in decreasing pollutant bioavailability varies in different soils. Furthermore, the effects of biochar on

PAHs bioaccumulation vary with the molecular weight of PAHs as well. In the study of Gomez-Eyles et al. (2011), biochar application reduced the total and bioavailable PAHs in soil and the accumulation of total PAHs in earthworms (up to 45%). However, the earthworm accumulation of lighter PAHs (2- and 4-ring) increased, while that of heavier and more toxic PAHs decreased (more than 20% by day 28), which may be attributed to the high affinity of heavier PAHs to biochar (Northcott and Jones, 2001).

4.1.3 Potential of biochar in removing toxic metabolites released by earthworms

The phenomenon that earthworms release toxic metabolites after metabolic conversion of low molecular weight PAHs in their bodies has been reported in previous studies, implying secondary pollution caused by PAHs metabolism in earthworms. Schmdit et al. (2017) found that earthworms released phase II conjugates, the metabolic products of phenanthrene and pyrene, into the soil environment. These persistence toxic metabolites are highly soluble in water and will pose a threat to environmental and human health once leached into groundwater (Schmdit et al., 2017). Another earlier study reported that anthracene was transformed into the more toxic 9,10-anthraquinone through earthworm metabolism (Coutiño-González et al., 2010). Different from anthracene, whose carcinogenicity is not yet known, anthraquinone has been found to induce liver tumors and kidney diseases in mice and classified as a 2B carcinogen by the International Agency for Research on Cancer (IARC) of the World Health Organization. Therefore, the release of toxic metabolites by earthworms after absorption of low molecular weight PAHs deserves more attention, even though Coutiño-González et al. (2010) found that the concentration of toxic metabolites decreased at the later stage of the experiment. PAH-degrading microbes (e.g., *Sphingomonas*.g and *Alphaproteobacteria*.c) (Li et al., 2020b) enriched and sheltered by biochar can use the adsorbed PAHs as a sole carbon source for growth, thereby degrading the PAHs (Widada et al., 2002; Li et al., 2020b). Adsorption and degradation of toxic earthworm metabolites on biochar is a safe and effective way to remove them from soil. However, there is limited information on other ways to eliminate toxic earthworm metabolites and the effects of biochar application on such metabolites, especially the metabolic transformation of the more toxic high molecular weight PAHs. More attention and research should be given to this scientific area in the future in order to comprehensively evaluate the combined effects of biochar and earthworms on soils contaminated with PAHs and the potential environmental risks.

4.1.4 Negative effects of biochar on earthworms and corresponding measures

The positive effects of biochar on the soil environment and

PAHs biodegradation have been widely reported. Negative effects of biochar application are usually related to feedstock property, biochar application rate, and soil properties (Chan et al., 2008; Liesch et al., 2010; Weyers and Spokas, 2011; Malev et al., 2016; Gong et al., 2018). The earthworms *Metaphire guillelmi* and *Amyntas corrugatus* have recently been proven to be capable of mineralizing bacterial and fungal cells for carbon and energy (Shan et al., 2010), which has positive effects on the redistribution and stabilization of soil organic matter (Haynes et al., 2003; Simpson et al., 2007; Miltner et al., 2012). But simultaneously, as accompanied effects, the PAHs adsorbed on biochar could have negative effects on earthworms through the microorganisms participating in the biodegradation of PAHs (Shan et al., 2013). The toxic effects of biochar on earthworms can be attributed to the following three aspects: (1) the toxins present in biochar, (2) the high pH of biochar, and (3) the reduction in water and nutrients available to earthworms (Van Zwieten et al., 2010; Jakob et al., 2012; Godlewska et al., 2021).

It is worth noting that the negative influences are mainly attributed to the bioavailable contaminants, which are not stably bound by biochar and thereby can be absorbed and metabolized by microorganisms, animals (Kelsey et al., 1997; Tang et al., 1998), and plants (Alexander, 1995; 2000). This is why the bioavailability of pollutants adsorbed on biochar and their toxic effects have attracted more and more scientific interest in recent years (Spokas et al., 2011; Hale et al., 2012; Hilber et al., 2017). If we pay some more attention to harmful substances which would potentially pollute the soil and its bio-system with biochar input, such as volatile organic compounds, perfluorochemicals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and other persistent organic pollutants, even certain heavy metals could be brought from the raw material of biochar and could also be produced in the pyrolytic process (Buss and Mašek, 2014; Kim et al., 2015; Weidemann et al., 2018; Antoniadis et al., 2019). PAHs have been commonly found in various biochar (Buss et al., 2016; Weidemann et al., 2018). Their concentrations in biochar, ranging from 0.08 mg kg⁻¹ (Freddo et al., 2012) to 172 mg kg⁻¹ (Khalid and Klarup, 2015), are affected considerably by feedstock type and pyrolysis conditions. In general, medium temperature (400–600°C), slow pyrolysis, and long residence time, as well as the use of plant material containing few PAHs precursors as feedstock can help to minimize PAHs in biochar (Hale et al., 2012; Wang et al., 2017b; Kończak et al., 2019; Godlewska et al., 2021). In more than 50 biochars produced under different pyrolysis conditions, Hale et al. (2012) found lower levels of dioxin and PAHs in the slow pyrolysis biochars, with the concentrations of bioavailable dioxin below the detection limit and those of bioavailable PAHs ranging from 0.17 ng L⁻¹ to 10.0 ng L⁻¹. Zielińska and Oleszczuk (2016) reported low concentrations of free PAHs in sewage sludge biochar produced at various pyrolysis temperatures. Pollutant content in biochar varies greatly and the amount of pollutants

adsorbed on biochar depends on interaction time (Qian and Chen, 2013; Ghaffar et al., 2015). With the aging of biochar, hydrophilic functional groups on its surface increase (Moreno-Castilla et al., 1995; Ghaffar et al., 2015), leading to the release of pollutants into the environment and the increase of toxic effects on soil organisms (Anyanwu et al., 2018; Kavitha et al., 2018).

Biochar may have negative effects on the growth and reproduction of earthworms and may even cause DNA damage, which may be attributed to the increased pH and decreased available water and nutrients with the addition of biochar (Cui et al., 2009; Zhang et al., 2019). Specific effects depend on the biochar properties, biochar application rate, and time. Biochar applied at an appropriate rate and for a suitable period has positive effects on the survival, growth, and reproduction of earthworms (Cui et al., 2009; Denyes et al., 2012; Malińska et al., 2016; Gong et al., 2018). In the study of Li and Alvarez (2011), earthworm cocoon production was not affected when biochar was applied at 0.5% but was significantly reduced when biochar was applied at 5%. Similarly, Zhang et al. (2019) found that the average weight of *Eisenia fetida* increased significantly at low biochar rate (1%–3%) but decreased significantly at high biochar rate (10%). Therefore, the negative effects of biochar addition on earthworms can be avoided or minimized by applying the right biochar at an appropriate rate.

4.2 Earthworm-assisted PAHs-contaminated soil remediation using biochar and its limitations

4.2.1 Potential of earthworms in promoting the adsorption and degradation of PAHs on biochar

The deep soil layers, which are important for soil texture, aeration, and microbial activity, could be influenced by biochar incorporation and its mobility in soil (Leifeld et al., 2007). Earthworm activities create biopores in the soil profile, and these pores may be interconnected laterally with each other in the drilosphere (Pagenkemper et al., 2015). The large ingestion capacity of geophagous earthworms (Lavelle, 1988; Brown et al., 2000) play a significant role in the migration and redistribution of the ingested matters, which is a bioturbation process (Hindell et al., 1997; Garcia and Fragoso, 2002). Under the bioturbation of earthworm *Pontoscolex corethrurus*, charcoal was found to be well mixed with soil (Topoliantz et al., 2002; Topoliantz and Ponge, 2003). According to the research of Bergknut et al. (2007), the bioturbation of earthworm *Pontoscolex corethrurus* resulted in the mobilization and incorporation of biochar in the forest soils (Alfisols and Mollisols) of Central Europe. Earthworm bioturbation enhances the mobility of biochar in soil and leads to closer contact between biochar and contaminated soils, which ultimately increases the adsorption of pollutants on biochar.

Furthermore, bioturbation also significantly influences the

activities of microorganisms and extracellular enzymes (Sanchez-Hernandez et al., 2019). The adsorption of enzyme molecules on the hydrophobic surface (low C/N ratio) and in the pores of biochar may lead to enzyme activity decrease (Chintala et al., 2014). The functional groups such as hydroxyl and carboxyl on biochar surface may interact with the amino group of enzyme molecule and thus reduce enzyme affinity to substrates (Khadem and Raiesi, 2019). Recent studies have shown that earthworms could improve the activities of biochar-adsorbing enzymes, which may be related to the gastrointestinal and external mucus secreted by earthworms (Sanchez-Hernandez, 2018). The activities of carboxylesterase, β -glucosidase, alkaline phosphatase, and arylsulfatase of biochar particles increased after earthworm introduction (Sanchez-Hernandez, 2018). Interestingly, carboxylesterase-coated biochar was found to have a high affinity to some highly toxic pesticide metabolites (Wheelock et al., 2008). However, more research is needed on earthworm-assisted remediation of PAHs-contaminated soils using biochar to answer such questions as whether the activities of PAHs-degrading enzymes on biochar surface are stimulated by earthworm introduction and what are the related mechanisms.

4.2.2 Negative effects of earthworms on biochar

The biodegradation of PAHs is strongly influenced by soil properties such as soil pH and organic matter content, specially the former. Generally, slightly acidic and alkaline soil conditions are favorable for microbial degradation of PAHs (Emoyan et al., 2018). Earthworms have been found to lower soil pH by releasing organic acids, while biochar is more likely to increase soil pH, which should be paid attention to when soil pH is an important influencing factor of pollutant transformation (Huang et al., 2020). Sorption to soil particles and dissolved organic matter (DOM) greatly affects the migration and degradation of PAHs (Weber et al., 1992; Pignatello and Xing, 1995). Garau et al. (2022) found that earthworms accelerated the degradation of biochar organic matter and increased DOM content in soil. DOM may compete with soil matrix for PAHs and increase the bioavailable PAHs in soil, weakening the remediation effect of biochar (Ling et al., 2005). In a word, much remains to be explored about the effects of earthworms on PAHs-contaminated soil remediation using biochar, and relevant research is warranted in the future.

5 Conclusion

It is urgent to solve the problem of PAHs pollution of soils. Both earthworm introduction and biochar application have been proved to be effective in PAHs-contaminated soil remediation. Earthworms activities (e.g., feeding, burrowing, and casting) improve soil texture, structure, and nutrient

status, creating a better soil environment for soil microbes and consequently accelerating PAHs biodegradation. The high porosity and large specific surface area of biochar endow it a strong ability to adsorb and immobilize PAHs. Furthermore, it was shown that biochar addition in PAHs-contaminated soils reduced PAHs in soil solution and PAHs accumulation in earthworms. In addition, the PAHs-degrading bacteria in earthworm gut are stimulated, reducing pollutant transfer along the food chain. Meanwhile, earthworm bioturbation enhances biochar migration in soil and the activities of enzymes on biochar surfaces. Although the growth and reproduction of earthworms may be negatively affected by biochar, these negative effects can be eliminated, or at least diminished to a negligible level, by choosing the right pyrolysis conditions for biochar production and applying biochar at an appropriate rate. Considering the lack of studies on PAHs removal effects of biochar-vermiremediation and the underlying mechanisms, future studies are needed to better evaluate the physicochemical and biological interactions among biochar, earthworms, and PAHs in soil.

Acknowledgements

This study was financially supported by the National Natural Science Foundation of China (Grant No. 41201305), the National Science and Technology Fundamental Resources Investigation Program of China (Grant No. 2018FY100300), Guangdong Natural Science Foundation (Grant No. 2021A1515011543) along with Guangdong Provincial Agricultural Science and Technology Development and Resources and Environment Protection Management Project (Grant No. 2022KJ161).

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