Influence of biochar particle size on ^{14}C-phenanthrene extractability and mineralisation in soil

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Abstract

Biochar can influence the bioaccessibility and biodegradation of polycyclic aromatic hydrocarbons (PAHs) in soil, but the role of biochar particle size in this process remains largely underexplored. In this study, soil was spiked with ^{12}C- & ^{14}C-phenanthrene and subsequently amended with biochar of <0.6 mm and 2 – 4 mm particle size at 0.0%, 0.1%, 1.0%, and 10.0%, respectively. The amended soils were aged for 60 d and ^{14}C-phenanthrene extractability and mineralisation were monitored at 1 d, 15 d, 30 d, 45 d, and 60 d. The total residual ^{14}C-activity and extractable fractions reduced over time with increasing biochar amounts irrespective of biochar particle size. Similarly, longer lag phases, slower rates, and lower extents of mineralisation were observed over time with increasing biochar amounts. Solvent extractability and reduction in residual ^{14}C-activity were higher in <0.6 mm amended soils, which were attributed to a higher surface area and shorter diffusion pathway. Hydroxypropyl-β-cyclodextrin (HP-β-CD) extracted ^{14}C-phenanthrene correlated with the extents of mineralisation with stronger agreement in <0.6 mm amended soils (R^2 = 0.77 – 0.86) than in 2 - 4 mm amended soils (R^2 = 0.57 – 0.82). The weakest correlation was observed at 10.0% of 2 - 4 mm. This study demonstrated HP-β-CD's potential for predicting phenanthrene microbial degradation in biochar-amended soils and highlighted the influence of biochar particle size on phenanthrene bioaccessibility and biodegradability. These findings are important for mitigating phenanthrene risks in soil and optimising its sorption stability using biochar.

Keywords: bioaccessibility, biochar, cyclodextrin, mineralisation, polycyclic aromatic hydrocarbon.

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic chemicals with two or more benzene rings (Lawal, 2017). They can be produced from the incomplete combustion of fossil fuels or organic matter (Quilliam et al., 2013; Lawal, 2017). They are considered to be persistent organic pollutants (POPs) as they display persistent, toxic and bioaccumulatory properties and pose risks to human and environmental health (Wu et al., 2019; Patel et al., 2020). PAHs are hydrophobic and can sorb to soil, making soil a sink for PAHs in the environment (Okere and Semple, 2012). PAHs may also have negative impacts on soil health and function; however, organic amendments can be used to reduce these impacts of PAHs by reducing PAH mobility and bioaccessibility (Puglisi et al., 2007; Sigmund et al., 2018; Kaur and Sharma, 2020). PAHs have different molecular weights, ring numbers, and structural complexity which influence their hydrophobicity, bioaccessibility, biodegradation, and environmental persistence (Riding et al., 2013). As a result, PAHs with high molecular weight, ring number, and complex structure are more recalcitrant to biodegradation and persist more in the environment (Stroud et al., 2007; Papadopoulos, Reid et al., 2007; Baldantoni et al., 2017). Soil-indigenous microbes can biodegrade PAHs, but susceptibility to biodegradation is influenced by their bioavailability (Reid et al., 2001). Thus, the biodegradation of most PAHs in soil is not limited by microbial population or catabolic ability but by their desorption and bioavailability (Semple et al., 2006; Allan et al., 2007).

Recently, environmental remediation strategies have moved towards more sustainable approaches, where possible (Patel et al., 2020). The use of biochar as an amendment for PAH-contaminated soil, is cost-effective, environmentally friendly, and sustainable (Zahed et al., 2021; Guo et al., 2021; Guo et al., 2022). Biochar has been demonstrated to improve soil physicochemical properties and soil

63 microbial activities thereby improving PAH biodegradation (Kong et al., 2021). Biochar can also
64 mitigate the impact of PAHs in the soil through sorption by transferring PAHs in the soil to biochar
65 thereby reducing the leaching and bioaccessibility of PAHs in soil (Chen & Yuan, 2011; Ogbonnaya
66 et al., 2014; Jimenez et al., 2018). Reduction in bioaccessibility reduces potential exposure thereby
67 mitigating possible toxicity, and the bioaccesible concentration of a PAH is of interest in risk
68 assessment as it controls potential biodegradation and toxicity (Riding et al., 2013; Semple et al.,
69 2013; Umeh et al., 2017). Non-exhaustive extraction techniques (NEETs) have been demonstrated
70 as effective for predicting the concentration of PAHs available for microbial degradation (e.g.
71 Alexander & Kelsey, 1997; Reid et al., 2000; Rhodes et al., 2008a; Rhodes et al., 2008b; Semple et
72 al., 2013; Cachada et al., 2014; Oyelami et al., 2014; Ogbonnaya et al., 2016; Cao et al., 2022). In this
73 study, hydroxypropyl-β-cyclodextrin (HP-β-CD) solvent extraction (a NEET) was used to predict ^{14}C-
74 phenanthrene microbial degradation because it has been demonstrated as an efficient, reliable, and
75 time-effective technique for predicting PAH microbial degradation in soil (e.g., Patterson et al.,
76 2004; Swindell & Reid, 2006; Semple et al., 2006; Papadopoulos et al., 2007; Bernhardt et al., 2013;
77 Ogbonnaya et al., 2014; Adedigba et al., 2018; Leech et al., 2020; Vázquez-Cuevas et al., 2021;
78 Posada-Baquero et al., 2022; Jin et al., 2023).

79

80 Biochar improves the sorption of PAHs in soil which is dependent on the properties of biochar, soil,
81 PAHs, and soil-biochar contact duration (Zhang et al., 2010). Studies have demonstrated that HP-β-
82 CD extraction and microbial degradation in soil amended with black carbon reduces with an increase
83 in contact time and amendment amount (Rhodes et al., 2008a; Rhodes et al., 2010; Rhodes et al.,
84 2012; Ogbonnaya et al., 2014a; Ogbonnaya et al., 2014b, Oyelami et al., 2014; Oyelami et al., 2015;
85 Ogbonnaya et al., 2016). Additionally, desorption could predict PAH mineralisation in black carbon
86 amended soils (Oyelami et al., 2014; Ogbonnaya et al., 2014a; Ogbonnaya et al., 2016; Yu et al.,

2016), but desorption could not predict mineralisation at higher black carbon amounts (Rhodes et al., 2008a; Rhodes et al., 2012). This raises concern about the suitability of HP-β-CD extraction for predicting the mineralisation of PAHs in soils amended with black carbon. In addition, phenanthrene biodegradation exceeded solvent extraction in past studies (Rhodes et al., 2008a; Rhodes et al., 2012; Ogbonnaya et al., 2016) though phenanthrene desorption has been reported to be higher than mineralised (Oyelami et al., 2014; Kang et al., 2019). There is notable information in the literature regarding the sorption and desorption behaviour of biochar and a disparity in the impact of biochar particle size on PAH mineralisation. Fine particle biochar has been shown to demonstrate higher sorption capacities and rates compared to coarse particle biochar (Kang et al., 2018; Kang et al., 2019; Jin et al., 2022; He et al., 2022). However, it has also exhibited higher desorption for phenanthrene (Kang et al., 2019), ammonium nitrogen (He et al., 2022), and phosphorus (Sarfraz et al., 2020). Additionally, while powdered biochar (<250 μm) showed greater phenanthrene mineralisation than raw biochar (<2–4 mm) (Kang et al., 2019), contradictory findings indicate that coarse biochar (3–7 mm) resulted in higher phenanthrene mineralisation than fine biochar (≤2 mm) (Ogbonnaya et al., 2014a; Ogbonnaya et al., 2014b). These conflicting results warrant further investigation. Therefore, we hypothesized that phenanthrene extractability and mineralisation kinetics in soil are mediated by the particle size of biochar, which influences sorption – desorption dynamics due to differences in surface area, particle number, and diffusion pathway.

Although studies have demonstrated that fine particle biochar can improve microbial activity and abundance in soil (Chen et al., 2017; Sarfraz et al., 2020; Zhao et al., 2020; Özenç et al., 2023), and smaller particle-sized biochar can enhance soil pH and water holding capacity, influencing the mobility and accessibility of PAHs (Chen et al., 2017; Liao & Thomas, 2019; Sarfraz et al., 2020), there remains insufficient information on how biochar particle size affects PAH extractability and

mineralisation. While it is widely accepted that fine biochar particles have higher sorption capacities (Kang et al., 2018; Kang et al., 2019; Jin et al., 2022; He et al., 2022), most studies focus on the impact of biochar particle size on soil physicochemical properties (e.g., Singh et al., 2010; Yao et al., 2012; Jin et al., 2016; Pratiwi et al., 2016; Xu et al., 2016; Esmaeelnejad et al., 2017; Lim et al., 2017; Lim & Spokas, 2018; Billah et al., 2019; Duarte et al., 2019; Alghamdi et al., 2020; Edeh et al., 2021; Özenç et al., 2023). Therefore, this study investigated the effect of biochar particle size on ^{14}C-phenanthrene bioaccessibility and mineralisation, which are crucial for predicting phenanthrene fate and behaviour in soil, enhancing remediation strategies, and mitigating the long-term environmental and health risks of phenanthrene contamination in soil.

2 Materials and methods

2.1 Sample collection.

The soil sample was collected from Hillam Farm green energy site in Cockerham Lancaster, United Kingdom (latitude 53.97, longitude -2.84). Soil in this area is described as loamy and sandy soils with naturally high groundwater and peaty surface (LandIS Soilscapes viewer, 2025). The soil was air-dried, sieved through a 2 mm mesh to remove unwanted materials (stones, debris, etc.), and was stored at 4 °C. Biochar was purchased from a commercial source (SoilFixers), Royal Wootton Bassett, United Kingdom. The properties of the biochar and soil are shown in Table 1 and Table 2, respectively.

Table 1: Biochar properties and pyrolysis conditions as provided by the supplier (SoilFixers, UK). This describes the biochar and not the separated particle size fractions.

Parameter	**Value**
Feedstock	Hardwood logs
Pyrolysis equipment	Retort Kiln
Pyrolysis temperature	700 – 900 °C
Pyrolysis duration	5 – 12 h
Particle size	0 – 8 mm
pH	8.8 – 10
Volatiles	9 – 15%
Ash max.	3 – 6%
Moisture max.	8 – 12%
C Fixed	>76%

Table 2: Properties of the soil sample used in this study. Values are in mean ± SD except the values for sand, silt, and clay

Parameters	Value
Sand (%)	71.43
Silt (%)	26.19
Clay (%)	2.38
Soil texture	Sandy loam
pH	7.28±0.03
Electrical conductivity (μS cm^{-1})	382±4.36
Organic matter (%)	6.47±0.25
C:N	10.36±0.08
Total carbon (mg/kg)	102.41±4.44
Total organic carbon (mg/kg)	98.85±3.58
Inorganic carbon (mg/kg)	3.56±1.02
Ammonium nitrogen (mg/kg)	0.37±0.01

Nitrate nitrogen (mg/kg)	3.61±0.15

2.2 Soil spiking and amendment

Soil (2.1 kg; dw) was rehydrated to field moisture content (30%) and was spiked with ^{12}C and 9-^{14}C–phenanthrene (100 mg/kg and 3,800 DPM/g; dw) (Reid et al., 2000; Doick et al., 2003; Stokes et al., 2005). Soil (approx. ¼) was initially spiked using acetone as the carrier solvent at a 1:20 (v/w) solvent-to-soil ratio. This portion was left in a fume hood for 3 h to allow complete volatilisation of the solvent. The remaining soil was subsequently added in three equal portions, with thorough mixing after each addition to ensure uniform distribution of the contaminant and overall homogeneity. The spiked soil was mixed thoroughly in a glass bowl using a stainless-steel spoon (Doick et al., 2003). Soil (300 g; dw) was weighed into a clean glass bowl, rehydrated, and spiked with ^{12}C-phenanthrene as ^{12}C blank. Soil (300 g) for ^{14}C-blank was weighed into a clean glass bowl. The remaining soil was amended with biochar (<0.6 mm and 2 – 4 mm particle size) at 0.1 %, 1.0%, and 10%, respectively. The soils (amended and blank) were weighed (100 g; n = 3) into amber bottles, covered with loose Teflon–lined screw caps, to allow ambient oxygen exchange, and incubated in the dark at 21 ± 2 ℃ for 60 d. The incubated soils were sampled at 1 d, 15 d, 30 d, 45 d, and 60 d to measure the total residual ^{14}C-activity, solvent extractability, and mineralisation kinetics. Prior to each sampling, the soil field moisture condition (30%) was maintained with sterile distilled water by weighing the bottles and replacing lost moisture.

2.3 Determination of total ^{14}C – activity in the soil

Soil (1.0 g) from the treatment conditions, was weighed into a cellulose combustion cone and Combustaid (200 µl) was added to the soil (Reid et al., 2000; Doick et al., 2003; Stokes et al., 2004; Doick et al., 2005). The soil was combusted for 3 min using a sample oxidiser (Packard 307). The released $^{14}CO_2$ was trapped with 10 ml of CarbonTrap and was delivered into a 20 ml vial with 10 ml of CarbonCount as a scintillation fluid. The efficiency (>96%) of the sample oxidiser to trap the evolved $^{14}CO_2$ was determined before combustion. The 20 ml vial was stored in the dark for 24 h before quantifying for 10 min with a liquid scintillation counter (LSC; Canberra Packard Tri-Carb 2250CA).

2.4 Extractability of ^{14}C – phenanthrene in the soil

2.4.1 Dichloromethane (DCM) extraction of ^{14}C – phenanthrene

Soil (1.5 g) from the soil incubations, was weighed into a 50 ml Teflon-lined centrifuge tube. Anhydrous sodium sulphate (1.5 g) was added to the soil and 20 ml of DCM was added to the tube (Reid et al., 2000; Doick et al., 2003; Papadopoulos, Paton et al., 2007; Rhodes et al., 2008b). All tubes were tightly closed and placed in an orbital shaker at 100 RPM for 24 h. Afterwards, the tubes were centrifuged at 4000 RPM for 1 h. The supernatant (5 ml) was pipetted into a 20 ml vial, and 14 ml of Ultima Goldstar scintillation fluid was added to the vial. The vial was stored in the dark for 24 h before quantifying for 10 min using an LSC. The remaining supernatant was safely and carefully discarded.

2.4.2 Hydroxypropyl-β-cyclodextrin (HP-β-CD) extraction of ^{14}C – phenanthrene

Soil (1.5 g) from the soil incubations, was weighed into a 50 ml Teflon-lined centrifuge tube, and 25 ml of 50 mM HP-β-CD solution was added to the tube (Reid et al., 2000; Stokes et al., 2005; Doick et al., 2006; Papadopoulos, Reid et al., 2007). All tubes were sealed and placed on an orbital shaker at 100 RPM for 24 h. Afterwards, the tubes were centrifuged at 4000 RPM for 1 h. The supernatant (5 ml) was transferred into a 20 ml vial and 14 ml of Ultima Goldstar scintillation fluid was added to the vial. The vial was stored in the dark for 24 h before quantifying for 10 mins using an LSC. The remaining supernatant was properly discarded.

2.5 Respirometric monitoring of ^{14}C – phenanthrene mineralisation in soil

Soil (10 ± 0.2 g) from the soil treatment conditions, was weighed into a 250 ml modified Schott bottle and 30 ml of sterile MBS (1:3 soil – liquid ratio) was added to the soil to form a slurry (Reid et al., 2001; Doick et al., 2003; Allan et al., 2007). A 7 ml vial containing 1 ml of 1 M NaOH was attached to the bottle to trap evolved $^{14}CO_2$. The bottles were tightly closed, placed on an orbital shaker (100 RPM) and monitored at 21 ± 2 °C. The bottles were sampled at 3 h intervals for an initial 9 h, then daily for 14 d. At each sampling, the 7 ml vial was removed and replaced with a fresh vial. Then, 5 ml Ultima Goldstar scintillation fluid was added to the sampled vial. The sampled vial was stored for 24 h in the dark before quantifying using LSC.

2.6 Statistical analysis

The data was processed using MS Excel and analysed using IBM SPSS 28. Data plots were done using SigmaPlot. Data were analysed using a one-way analysis of variance (ANOVA), Tukey's HSD post hoc, independent sample t-test, and simple linear regression. The lag phases were calculated as the time

taken to mineralise 5% of the ^{14}C-phenanthrene, the fastest rates as the highest residual ^{14}C-phenanthrene mineralised per hour, and the extents of mineralisation as the cumulative ^{14}C-phenanthrene mineralised in 14 d. All were calculated from the residual activity at each contact time.

3 Results

3.1 Temporal changes in the total residual ^{14}C-phenanthrene activity in the amended soils

The total residual ^{14}C-phenanthrene activity in the amended soils was monitored at 1 – 60 d (Figure 1). The residual ^{14}C-activity is expressed as a percentage of the initial spiked ^{14}C-activity. Noticeably, the residual ^{14}C-activity in the amended soils reduced as the soil contact duration increased in <0.6 mm and 2 – 4 mm amendments (Figure 1). The decrease followed a similar trend irrespective of amendment particle size. However, the residual activity was lower in <0.6 mm amended soils. As expected, the residual ^{14}C-activity was higher at 1 d and lower at 60 d (all $p < 0.05$) compared to other contact times. In soils amended with <0.6 mm, the residual ^{14}C-activity at 0.1% biochar application was lower than in 1.0% ($p > 0.05$) and 10.0% ($p < 0.05$) at 60 d (Figure 1). The 10.0% biochar amount showed higher ($p < 0.05$) residual activity at 60 d compared to other amended soils in both particle size fractions. In soils amended with 2 – 4 mm, the residual ^{14}C-activity at 1.0% amendment was lower than in 0.1% ($p > 0.05$) and 10.0% ($p < 0.05$) at the end of incubation (Figure 1). Noticeably, at 15 d, 10.0% of 2 – 4 mm had only lost 1.77% of the initial spiked activity. Overall, at 60 d, <0.6 mm amended soil lost 61.64 – 75.91% of the initial spiked activity, while 2 – 4 mm amended soils lost 58.38 – 61.29% of the initial spiked activity.

3.2 Temporal changes in the extractability of ^{14}C-phenanthrene in amended soils

The extraction of ^{14}C-phenanthrene using DCM and HP-β-CD in the amended soils was measured over the 60 d incubation (Figure 2 and Figure 3). This was expressed as a percentage of the residual ^{14}C-activity at each sampling time point. Noticeably, DCM and HP-β-CD extractability of ^{14}C-phenanthrene decreased over time in soils amended with <0.6 mm and 2 – 4 mm (Figure 2; Figure 3). The DCM-extractable ^{14}C-phenanthrene was substantially higher than the HP-β-CD-extractable ^{14}C-phenanthrene in the amended soils regardless of particle size. The DCM-extractable ^{14}C-phenanthrene at 1 d was higher than that at 15 – 60 d, and that at 60 d was significantly lower than that at 1 – 45 d ($p < 0.05$). At the end of incubation, in soils amended with <0.6 mm biochar particle size fraction, the DCM-extractable ^{14}C-phenanthrene in soil amended with 10.0% biochar was lower than in other amended soil ($p < 0.05$), and the DCM-extractable ^{14}C-activity in soil amended with 1.0% biochar was lower ($p > 0.05$) than at 0.1% (Figure 2). Also, in soils amended with 2 – 4 mm, the DCM-extractable ^{14}C-phenanthrene at 10.0% was lower than other amended soils ($p < 0.05$), and the DCM extractable ^{14}C-activity at 1.0% was lower ($p > 0.05$) than at 0.1%. All biochar amended soils had lower DCM-extractable ^{14}C-activity than the control regardless of the particle size (Figure 2), which was significant ($p < 0.05$) for all 2 – 4 mm amended soils and significant ($p < 0.05$) for all <0.6 mm amended soils except soil amended with 0.1% biochar (Figure 2). Furthermore, at 60 d, in soils amended with <0.6 mm, HP-β-CD-extractable ^{14}C-phenanthrene at 10.0% was lower than at 1.0% ($p > 0.05$) and 0.1% ($p < 0.05$), and that at 1.0% amendment was lower ($p > 0.05$) than at 0.1% (Figure 3). In soils amended with 2 – 4 mm, the HP-β-CD-extractable ^{14}C-phenanthrene at 10.0% amendment was lower ($p < 0.05$) than at 1% and 0.1%, while there was significant ($p > 0.05$) difference between 1.0% and 0.1% (Figure 3). Generally, the reduction in solvent extractability increased with an increase in biochar amount in <0.6 mm and 2 – 4 mm amended soils. Additionally, <0.6 mm amended soils had higher extractability than 2 – 4 mm amended soils ($p > 0.05$).

3.3 Temporal changes in ^{14}C-phenanthrene catabolism in amended soils

The lag phases, rates, and extents of ^{14}C-phenanthrene mineralisation were monitored in the soil treatments from 1 – 60 d (Table 3; Figure 4; Figure 5). The mineralisation kinetics were calculated from the residual ^{14}C-activity at each sampling time point. Noticeably, the lag phases were longer, degradation rates were slower, and the extents of mineralisation were lower over time in <0.6 mm and 2 – 4 mm amended soils (Table 3, Figure 4, and Figure 5). In <0.6 mm amended soils, there were no significant differences between the lag phases at 1 d and 30 d ($p > 0.05$). The lag phases at other contact times were longer than at 15 d (Table 3; $p < 0.001$), with those at 60 d significantly longer than at 1–45 d ($p < 0.05$), and at 45 d longer than at 1–30 d ($p < 0.05$). The fastest rates at 15 d were more rapid than the rates at 1 d, 30 d, 45 d, and 60 d (Table 3; $p < 0.001$). The rates at 1 d were faster than at 30 - 60 d ($p < 0.01$), while rates at 30 d were faster than at 45 d ($p < 0.01$), with no significant difference between 45 and 60 d ($p > 0.05$). There were no significant differences in the extents of mineralisation at 1 d and 15 d ($p > 0.05$), but both contact times showed significantly higher extents of mineralisation than 30 – 60 d (Table 3; Figure 4; $p < 0.001$). The extents of mineralisation at 30 d were significantly higher than at 45 – 60 d ($p < 0.01$), but there were no significant differences between the extents of mineralisation at 45 d and 60 d (Figure 4; $p > 0.05$).

In <0.6 mm amended soils, significant differences ($p < 0.05$) were observed in lag phases in the amended soils on 1 d, 30 d, and 45 d, while there were no significant differences ($p > 0.05$) in lag phases at 15 d and 60 d. The lag phases ranged from 35.8 – 67.6 h on 1 d, 53.3 – 83.6 h on 30 d, and 184.3 – 192.6 h on 60 d. The control had shorter lag phases than amended soils at 45 d and 60 d. The lag phases in the amended soils were not significantly different at 60 d ($p > 0.05$), but all

amended soils showed longer lag phases than the control ($p < 0.001$). There was no significant difference in the fastest rates in amended soils from 15 – 60 d. The rates ranged from 0.3 – 0.8 $\%^{14}CO_2$/h on 1 d, 0.3 – 0.4 $\%^{14}CO_2$/h on 30 d, and 0.1 – 0.1 $\%^{14}CO_2$/h on 60 d. Significantly ($p < 0.05$) higher rates were observed in 0.1% at 1 d compared to other amended soils. The control displayed faster rates than all amended soils from 30 – 60 d. Significant differences ($p < 0.05$) were observed in the extents of mineralisation in the amended soils from 1 – 15 d, with no significant differences ($p > 0.05$) observed from 30 – 60 d. The extents of mineralisation ranged from 26.8 – 53.6% on 1 d, 23.1 – 26.2% on 30 d, and 9.0 – 9.5% on 60 d. The soil amended at 10.0% showed significantly ($p < 0.05$) lower extents of mineralisation at 1 d and had overall lower extents of mineralisation compared to other amendment levels. The amended soils showed significantly ($p < 0.05$) higher extents of mineralisation than the control from 15 – 30 d. The extents of mineralisation in the control at 60 d was higher than in all amended soils ($p < 0.05$).

In 2 – 4 mm amended soils, there were no significant differences in lag phases at 1 d and 15 d ($p > 0.05$), but the lag phases at 15 d were significantly shorter than 30 – 60 d ($p < 0.001$). There were no significant differences in lag phases at 30 – 60 d ($p > 0.05$). The lag phases ranged from 27.3 – 39.7 h on 1 d, 131.4 – 202.0 h on 30 d, and 140.7 – 202.9 h on 60 d. There were no significant differences in the rates at 1 d and 15 d ($p > 0.05$), but both contact times showed faster rates than 30 – 60 d (Table 3; $p < 0.001$). The rates at 30 d were faster than at 45 d ($p < 0.05$), and there were no significant differences in rates at 45 d and 60 d ($p > 0.05$). The rates ranged from 0.9 – 1.1 $\%^{14}CO_2$/h on 1 d, 0.2 – 0.3 $\%^{14}CO_2$/h on 30 d, and 0.1 – 0.2 $\%^{14}CO_2$/h on 60 d. The extents of mineralisation at 1 d were higher ($p < 0.001$) than at 15 d, and the extents of mineralisation at 15 d were higher ($p < 0.001$) than at 30 – 60 d (Table 3, Figure 5). There were no significant differences in the extents of mineralisation at 30 – 60 d ($p > 0.05$). The extents of mineralisation ranged from 45.5 – 69.5% on 1

309 d, 9.5 – 13.1% on 30 d, and 8.3 – 13.1% on 60 d. Significant ($p < 0.05$) difference in lag phases were
310 observed in the amended soils from 1 – 60 d. The control showed shorter lag phases than all
311 amended soils from 30 – 60 d. The soil amended at 10.0% showed longer lag phases than the control
312 from 1 – 60 d. At 15 d, the lag phases at 10.0% amendment were longer than in other soil treatment
313 conditions (Table 3; $p < 0.05$). At 30 d, the lag phases at 1.0% amendment were longer than in other
314 soil treatment conditions ($p < 0.05$). The control had significantly ($p < 0.05$ shorter lag phases at 60
315 d compared to amended soils. There were no significant ($p > 0.05$) differences in fastest rates in the
316 amended soils on 1 d and from 30 – 60 d. Significantly ($p < 0.05$) slower rates were observed in
317 10.0% at 15 d compared to other amended soils. The control showed higher rates than the amended
318 soils from 30 – 60 d. There were significant differences ($p < 0.05$) in the extents of mineralisation in
319 the amended soils from 1 – 15 d, but the differences in the extents of mineralisation from 30 – 60 d
320 were not significant ($p > 0.05$). At 1 d, the extents of mineralisation at 1.0% and 0.1% amendment
321 were significantly higher (Figure 5; $p < 0.001$) than at 10.0% amendment and in the control. Also, at
322 15 d, the extents of mineralisation at 10.0% amendment were significantly lower than in other soil
323 treatment conditions ($p < 0.001$). The soil amended at 10.0% showed significantly ($p < 0.05$) lower
324 extents of mineralisation than other amendment levels from 1 - 15 d, and had overall lower extents
325 of mineralisation compared to other amendment conditions. The differences in the extents of
326 mineralisation observed at 0.1% and 1.0% amendment from 1 – 60 d were not statistically significant
327 ($p > 0.05$).

328

329 At 1 d, 2 – 4 amended soils (0.1% and 1.0%) showed non-significant shorter lag phases ($p > 0.05$),
330 but significantly faster rates and higher extents of mineralisation ($p < 0.05$) compared to ≤0.6 mm
331 (0.1% and 1.0%) amended soils (Table 3, Figure 4, Figure 5). Soil amended with 2 – 4 mm at 10.0%
332 showed statistically significant ($p < 0.05$) shorter lag phases, faster rates, and greater extents of

mineralisation at 1 d compared to soil amended with <0.6 mm at 10.0%. At 15 d, <0.6 mm amended soils showed significantly ($p < 0.05$) shorter lag phases and faster rates than 2 – 4 mm amended soils. The extents of mineralisation in soil amended at 1.0% with <0.6 mm was not significantly ($p > 0.05$) higher than in soil amended at 1.0% with 2 – 4 mm at 15 d. However, the extents of mineralisation at 15 d in <0.6 mm (0.1% and 10%) amended soil were higher ($p < 0.05$) than in 2 – 4 amended soils (0.1% and 10%). At 30 d, <0.6 mm amended soils showed significantly shorter lag phases, faster rates, and higher extents of mineralisation compared to 2 – 4 mm treatments (Table 3; $p < 0.05$). The extents of mineralisation significantly reduced at 30 d in soils amended with 2 – 4 mm (Table 3). Noticeably, at 15 d, there was an obvious significant ($p < 0.05$) reduction in the extent of mineralisation (15.4%), with slower rate (0.3 $\%^{14}CO_2$/h), and longer lag phase (100.7 h) at 10.0% amendment for 2 – 4 mm (Table 3) compared to 10.0% of <0.6 mm at 56.3%, 1.1 $\%^{14}CO_2$/h, and 13.4 h, respectively. There was no significant ($p > 0.05$) difference in degradation rates from 30 – 60 d, and in the extents of mineralisation from 45 – 60 d in <0.6 mm and 2 – 4 mm amended soils (Table 3).

348 Table 3: Mineralisation kinetics for soils amended with <0.6 mm and 2 – 4 mm biochar at 0.0%, 0.1%,
349 1.0%, and 10.0%. The table shows lag phases, the fastest rates, and extents of mineralisation. Values
350 are in mean ± SEM (n = 3).

Contact time (days)	Particle size (mm)	Amendment amount (%)	Lag phase (h)	Fastest rate (%$^{14}CO_2$/h)	Extent of mineralisation (%)
1		0.0	37.88±1.62	0.63 ± 0.03	47.54 ± 2.28
	<0.6	0.1	35.78±5.65	0.83 ± 0.29	53.57 ± 4.52
		1.0	46.79±9.73	0.54 ± 0.26	41.31 ± 5.41
		10.0	67.58±9.62	0.33 ± 0.07	26.75 ± 3.69
	2 – 4	0.1	27.27±2.21	0.89 ± 0.02	69.54 ± 5.75
		1.0	29.10±4.01	1.12 ± 0.09	65.36 ± 5.55
		10.0	39.66±2.99	0.91 ± 0.17	45.50 ± 3.80
15		0.0	27.40±6.39	0.79 ± 0.15	40.52 ± 5.18
	<0.6	0.1	16.37±4.33	1.09 ± 0.13	52.62 ± 0.97
		1.0	12.78±3.33	1.27 ± 0.12	46.14 ± 3.58
		10.0	13.43±4.37	1.14 ± 0.21	56.30 ± 3.37
	2 – 4	0.1	30.28±5.67	0.70 ± 0.13	38.91 ± 3.95
		1.0	23.52±2.73	0.88 ± 0.05	42.02 ± 3.62
		10.0	100.71±15.03	0.31 ± 0.03	15.44 ± 2.24
30		0.0	75.28±8.46	0.42 ± 0.09	18.82 ± 1.86
	<0.6	0.1	53.25±4.93	0.44 ± 0.02	26.21 ± 2.35
		1.0	61.60±2.29	0.30 ± 0.11	24.15 ± 1.17
		10.0	82.58±29.73	0.31 ± 0.13	23.10 ± 4.33
	2 – 4	0.1	131.35±24.75	0.26 ± 0.07	13.12 ± 2.89
		1.0	201.95±49.13	0.14 ± 0.03	9.48 ± 2.65
		10.0	144.34±1.09	0.23 ± 0.03	10.83 ± 0.15
45		0.0	115.16±20.56	0.16 ± 0.01	15.84 ± 3.50
	<0.6	0.1	163.68±26.83	0.09 ± 0.03	11.05 ± 1.70
		1.0	124.01±12.19	0.14 ± 0.02	14.14 ± 0.95
		10.0	155.75±33.46	0.14 ± 0.01	11.76 ± 2.12
	2 – 4	0.1	165.20±39.04	0.15 ± 0.02	11.78 ± 3.34
		1.0	124.40±26.45	0.12 ± 0.04	15.26 ± 3.42
		10.0	142.60±21.06	0.16 ± 0.04	12.54 ± 2.02
60		0.0	98.74 ± 17.96	0.40 ± 0.05	16.86 ± 3.00
	<0.6	0.1	192.62±40.62	0.09 ± 0.03	9.48 ± 1.58
		1.0	183.45 ± 6.11	0.11 ± 0.04	8.97 ± 0.36
		10.0	184.32±13.58	0.22 ± 0.02	8.96 ± 0.68
	2 – 4	0.1	202.93±8.24	0.10 ± 0.01	8.27 ± 0.33
		1.0	140.74±37.75	0.23 ± 0.02	13.07 ± 2.83
		10.0	195.35±47.12	0.21 ± 0.01	9.98 ± 2.69

351

3.4 Relationship between HP-β-CD extracted ^{14}C-phenanthrene and extents of mineralisation

The relationship between the HP-β-CD extracted ^{14}C-phenanthrene and the extents of mineralisation was examined to check the suitability of HP-β-CD solvent extraction for predicting ^{14}C-phenanthrene microbial degradation in the biochar amended soils. The HP-β-CD extracted ^{14}C-phenanthrene correlated with the extents of mineralisation in the amended soil but this was influenced by the level of amendment and particle size. In <0.6 mm amended soils, at 0.1% amendment, HP-β-CD extracted ^{14}C-phenanthrene correlated with extents of mineralisation (R^2 = 0.86, slope = 0.99, intercept = -16.37). At 1.0% amendment, HP-β-CD extracted ^{14}C-activity (R^2 = 0.77, slope = 0.85, intercept = -1.22) also correlated with the extents of mineralisation, and at 10.0% amendment, HP-β-CD extracted ^{14}C-activity (R^2 = 0.82, slope = 0.65, intercept = 0.85) correlated with the extents of mineralisation. Similarly, in 2 – 4 mm amended soils, at 0.1% amendment, the HP-β-CD extracted ^{14}C-phenanthrene also showed a relationship with the extents of mineralisation (R^2 = 0.82, slope = 0.68, intercept = -20.06). Also, at 1.0% amendment, HP-β-CD (R^2 = 0.73, slope = 0.46, intercept = -7.01) correlated with the extents of mineralisation. Furthermore, at 10.0% amendment, HP-β-CD extracted ^{14}C-activity (R^2 = 0.57, slope = 0.54, intercept = -3.26) correlated with the extents of mineralisation.

4 Discussions

4.1 Changes in the total residual ^{14}C-phenanthrene in the amended soils

The initial recovery of spiked ^{14}C-phenanthrene ranged from 86.69% to 100.75%, meeting the acceptable variability threshold (<20%) required to validate spiking procedures (Doick et al., 2003). The reduction in the initial recovered ^{14}C-phenanthrene activity was attributed to volatilisation, adhesion to the blending vessel, and soil heterogeneity, which can create subsystems with varying contaminant concentrations (Doick et al., 2003). Additional factors such as sampling variation and potential microbial degradation within 1 d may have also contributed to reduced ^{14}C-activity. Indigenous soil microbes, capable of degrading PAHs, may play a significant role in this reduction, which is consistent with previous findings (Macleod and Semple, 2000; Doick et al., 2005 Couling et al., 2010; Okere et al., 2017). Comparisons between sterile and non-sterile soils further highlight the influence of soil microbiota on residual ^{14}C-activity (Macleod and Semple, 2000; Oyelami et al., 2014). Residual ^{14}C-phenanthrene activity decreased over time in both amended soils, with greater reductions observed in soils amended with <0.6 mm. This trend aligns with findings by Ogbonnaya et al. (2014a) that residual activity reduced in similar manner over time in soil in soil amended with ≤2 mm and 3 - 7 mm biochar. At a 10.0% biochar amendment level, residual ^{14}C-activity was higher regardless of particle size compared to 0.1% and 1.0%, indicating reduced ^{14}C-phenanthrene losses. The higher sorption capacity at 10.0% amendment minimized volatilisation and biodegradation (Rhodes et al., 2008a; Ogbonnaya et al., 2016; Bielska et al., 2018).

4.2 Changes in DCM and HP-β-CD extractability of ^{14}C-phenanthrene in the soils

The solvent extractability of ^{14}C-phenanthrene declined over time, with greater reductions observed at higher amendment levels regardless of biochar particle size. Extractability decreased

progressively, with the reduction at 60 d being more significant than at earlier time points (1 - 45 d). This aligns with studies showing that prolonged soil-PAH contact time enhances aging, leading to reduced PAH extractability in soil (Alexander & Kelsey, 1997; White et al., 1997; Macleod and Semple, 2000; Doick et al., 2005; Bielská et al., 2013). The reduction in solvent extractability of ^{14}C-phenanthrene was greater in the 10.0% amendment than in the 1.0% and 0.1% amendments. This supports previous findings that extractability decreases as black carbon content increases (Rhodes et al., 2008a; Ogbonnaya et al., 2014a; Yu et al., 2016; Ogbonnaya et al., 2016). Biochar addition resulted in reduced HP-β-CD extractability, with more significant effects at 5% and 10% amendments compared to 1% in soils amended with ≤2 mm and 3 - 7 mm biochar (Ogbonnaya et al., 2014a). The DCM extraction yielded higher ^{14}C-phenanthrene activity than HP-β-CD in all amended soils, which is consistent with the fact that HP-β-CD is a non-exhaustive extraction solvent (Reid et al., 2000; Semple et al., 2007). As noted by Doick et al. (2003), neither contaminant concentration nor spiking procedure significantly affects DCM or HP-β-CD extractability. Therefore, the observed differences in extracted ^{14}C-activity were attributed to aging, soil heterogeneity, biochar particle size, and biochar amount as these factors have been demonstrated to affect extractability in past studies (Alexander & Kelsey, 1997; Macleod & Semple, 2000; Doick et al., 2003; Doick et al., 2005; Rhodes et al., 2008a; Ogbonnaya et al., 2014a; Ogbonnaya et al., 2016).

Biochar's sorption capacity can be influenced by pore size, particle size, and surface area (Zhang et al., 2010; He et al., 2022). Fine particle biochar has a higher surface area and sorption capacity than coarse biochar (He et al., 2022). Smaller particles improved phenanthrene sorption rate and capacity (Kang et al., 2018), likely due to more exposed pores per unit surface area. In this study, soils amended with fine particle biochar (<0.6 mm) showed higher solvent extractability and reduced residual ^{14}C-activity. This may be due to the larger surface area and shorter diffusion pathways of

smaller biochar particles, which provide more improved sorption but enhance microbial degradation through faster desorption. Smaller biochar particles offer a larger surface area, providing more sites for phenanthrene sorption and microbial colonisation compared to larger particles. However, smaller particles have shorter diffusion pathways for phenanthrene movement, allowing faster sorption but also faster desorption, while larger particles have longer diffusion pathways likely due to longer pores and slower intraparticle diffusion, therefore slowing sorption rate and the desorption of sorbed phenanthrene. These differences imply that smaller particles not only enhance the efficiency of phenanthrene sorption but can also support more effective microbial degradation by providing better microbial accessibility and faster desorption of phenanthrene. Recent studies support these assumptions (Kang et al., 2018; Kang et al., 2019; Sarfraz et al., 2020; Jin et al., 2022; He et al., 2022). The increased surface contact of fine particle biochar with water or solvents enhances desorption (Sarfraz et al., 2020). Additionally, the interaction of the biochar with soil organic matter may result in the blockage of biochar pores and sorption sites (Liu et a., 2018), and this is higher in smaller particle size fraction due to higher surface area. This could reduce the sorption capacity of the biochar resulting in more phenanthrene been reversibly sorbed to soil organic matter thereby increasing the extractable fraction. However, if sorption site coating and pore blockage occur after contaminant sorption, they may hinder desorption and reduce extractability. Furthermore, dissolved organic carbon (DOC) increases the desorption of phenanthrene in soil resulting to greater extraction (Lou et al., 2019). This effect could also be higher in <0.6 mm amended soils as it has been reported that smaller particle size biochar releases more DOC in soil compared to higher particle size (Liu et al., 2016). Therefore, despite the higher sorption efficiency of fine particle biochar, coarse biochar may offer better long-term sorption stability.

444 **4.3 Changes in ^{14}C-phenanthrene mineralisation in the amended soils**

445

446 Soil-PAH contact time affects PAH bioavailability and catabolism (Macleod and Semple, 2000; Reid
447 et al., 2001; Wu et al., 2013; Wu et al., 2014; Omoni et al., 2020). In this study, ^{14}C-phenanthrene
448 catabolism decreased with an increase in contact time. This is consistent with the findings in earlier
449 studies (Rhodes et al., 2008a; Ogbonnaya et al., 2014a; Ogbonnaya et al., 2014b; Ogbonnaya et al.,
450 2016; Omoni et al., 2020). Similarly, the amount of biochar added to the soils also influenced ^{14}C-
451 phenanthrene mineralisation. Higher amounts of biochar (e.g. 10.0%) led to reduced mineralisation
452 irrespective of biochar particle size. This is consistent with earlier findings where increase in
453 amounts of black carbon caused a reduction in extents of mineralisation (Rhodes et al., 2008a;
454 Ogbonnaya et al., 2014a; Oyelami et al., 2015, Ogbonnaya et al., 2016; Bielska et al., 2018). This is
455 likely due to PAH sorption and decreased bioavailability (Ogbonnaya et al., 2016). Additionally,
456 considering the reduced mineralisation with increase in contact time and biochar amounts, over
457 time and under higher biochar amount, phenanthrene could become increasingly associated with
458 soil organic matter, mineral surfaces, and biochar, or diffuse into soil and biochar pores making it
459 inaccessible to soil microbes. These processes limit the fraction of phenanthrene available for
460 microbial degradation. Therefore, as bioavailable phenanthrene declines, microbial populations
461 may experience reduced substrate exposure, leading to the downregulation of key catabolic
462 enzymes involved in phenanthrene degradation.

463

464 In this study, higher mineralisation was observed in <0.6 mm amended soils compared to 2 – 4 mm
465 at 15 – 30 d. This is consistent with the reports of Kang et al. (2019) who reported higher
466 phenanthrene biodegradation with powdered biochar (<250 μm) compared to raw biochar (<2 mm).
467 In contrast, Ogbonnaya et al. (2014a) reported higher extractability and extents of mineralisation in

soil amended with biochar of 3 – 7 mm particle size fraction compared to ≤2 mm amended soil. However, the biochar properties may vary due to differences in feedstock, pyrolysis conditions, and state of weathering (Semple et al., 2013). For instance, powdered sewage sludge biochar (<250 μm) showed substantially higher phenanthrene desorption than raw sewage sludge biochar (<2 mm) but the difference in powdered and raw rice husk biochar was negligible (Kang et al., 2019). This was attributed to difference in chemical structure (aromaticity) of sewage sludge biochar and rice husk biochar. Over time, biochar of different particle size fractions but from same feedstock and pyrolysis conditions may offer similar effect on phenanthrene mineralisation as similar lag phases, degradation rates, and extents of mineralisation were observed in <0.6 mm and 2 – 4 mm amended soil from 45 d in this study. Generally, the lag phases and extents of mineralisation in soil amended with 10.0% of 2 – 4 mm biochar particle size fraction were respectively longer and lower than the control at 1 – 60 d. Ogbonnaya et al. (2014b) reported non-significant lower extents of mineralisation at 1.0% amendment compared to the control in ≤2 mm and 3 – 7 mm amended soils. Also, in soils amended at 1.0% using black carbon, the control showed better rates and extents of mineralisation than amended soils (Yu et al., 2016). Therefore, ≤1.0% biochar amendment could be more beneficial if the objective is to enhance contaminant biodegradation.

4.4 Relationship between HP-β-CD extractability and microbial degradation

According to Semple et al. (2007), the bioaccessible concentration provides a more realistic description of the microbial degradation endpoint for an organic contaminant in soil. Over two decades, non-exhaustive extraction using HP-β-CD has been demonstrated as a robust and reliable predictor of PAH microbial availability in soil (Reid et al., 2000; Stokes et al., 2005; Doick et al., 2006; Papadopoulos et al., 2007; Rhodes et al., 2008b; Ogbonnaya et al., 2014; Adedigba et al., 2018;

Vázquez-Cuevas et al., 2021; Posada-Baquero et al., 2022; Jin et al., 2023). HP-β-CD extraction has been demonstrated to be suitable for predicting microbial degradation of PAHs in soil amended with black carbon (Rhodes et al., 2008a; Ogbonnaya et al., 2014a; Oyelami et al., 2014; Ogbonnaya et al., 2016; Yu et al., 2016). HP-β-CD extraction predicts the extents of mineralisation better than DCM extraction as DCM extraction overpredicts microbial degradation (Reid et al., 2000; Doick et al., 2003; Papadopoulos, Paton et al., 2007; Adedigba et al., 2018). In this study, the HP-β-CD extracted ^{14}C-phenanthrene showed a strong association with the extents of ^{14}C-phenanthrene mineralisation, which is consistent with earlier reports (Wu et al., 2013; Adedigba et al., 2018; Vázquez-Cuevas et al., 2021; Posada-Baquero et al., 2022). However, the correlation was lower in 2 – 4 mm amended soils (R^2 = 0.82 – 0.57) than in <0.6 mm amended soils (R^2 = 0.86 – 0.77), especially at 10.0% of 2 – 4 mm amendment. Linear regression revealed a strong relationship between HP-β-CD extracted and total mineralisation at 0.1% amendment (R^2 = 0.67, slope = 0.95), but the R^2 and slope for 0.5 – 5% activated carbon amendment ranged from 0.51 – 0.13 and 2.19 – 12.73 respectively, indicating that HP-β-CD underpredicted total mineralisation at higher amendment (Rhodes et al., 2008a). Similar findings with a weaker correlation after 0.1% amendment were reported by Rhodes et al. (2012). Ogbonnaya et al. (2014a) demonstrated that HP-β-CD extraction was in good agreement with the extents of mineralisation in ≤2 mm and 3 – 7 mm biochar amended soils, but the correlation was better in ≤2 mm biochar compared to 3 – 7 mm biochar. Therefore, the amount and particle size of biochar influence the efficiency of HP-β-CD extraction for prediction microbial degradation of phenanthrene.

5 Conclusions

The higher extractability and mineralisation observed in soils amended with <0.6 mm biochar particles are attributed to higher surface area and shorter diffusion pathways, which facilitate easier desorption and greater bioavailability for microbial degradation. Conversely, larger biochar particles (2–4 mm) exhibit longer diffusion pathways, reducing desorption and bioavailability. Therefore, larger biochar particles may offer better sorption stability in the long-term. This study has also demonstrated that HP-β-CD extraction can effectively predict microbial degradation of ^{14}C-phenanthrene in biochar-amended soils. However, this prediction is influenced by biochar amount and particle size. This presents a challenge in risk assessment of biochar amended PAH-contaminated soil in predicting microbial degradation endpoint. These findings are important as they highlight the critical role of biochar particle size and amount in determining PAH desorption dynamics, bioaccessibility, and microbial degradation in contaminated soils which are critical for mitigating the adverse impacts of PAHs in soil.

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