Environments **2014**, *1*, 60-74; doi:10.3390/environments1010060

OPEN ACCESS

environments

ISSN 2076-3298

www.mdpi.com/journal/environments

Article

Influence of Wood Biochar on Phenanthrene Catabolism in Soils

Uchenna Ogbonnaya [1,2,*], Ayodeji Oyelami [2,†], Justin Matthews [2,†], Olusoji Adebisi [2,3] and Kirk T. Semple [2]

[1] EcoSystems Environmental Management Services Ltd., 8 Rasheed Alaba Williams Street, Off Admiralty Way, Lekki Peninsula, Lagos, Nigeria

[2] Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK; E-Mails: dejioyelami@yahoo.com (O.A.); justin.a.matthews@gmail.com (J.M.); k.semple@lancaster.ac.uk (K.T.S.)

[3] Section for Integrative Bioenergetics Ecotoxicology and Environmental Systems, Department of Microbiology, Faculty of Life Sciences, University of Ilorin, Ilorin P.M.B. 1515, Nigeria; E-Mail: oo.adebisi@outlook.com

† These authors contributed equally to this work.

* Author to whom correspondence should be addressed; E-Mail: uchenna.ogbonnaya@eemsltd.com or o_rule@hotmail.com; Tel.: +234-817-884-4908.

Received: 30 June 2014; in revised form: 4 August 2014 / Accepted: 5 August 2014 / Published: 12 August 2014

Abstract: The impact of increasing amendments of two particle sizes of biochar (≤2 mm and 3–7 mm), applied at 0%, 0.01%, 0.1% and 1% concentrations, on the development of indigenous phenanthrene catabolism was investigated in two soils with different soil organic matter contents. Mineralisation of ^{14}C-phenanthrene was measured after 1, 20, 60 and 100 d soil-phenanthrene-biochar aging period. The presence of biochar in the pasture soil (low OM) resulted in a decrease in the lag phase of ^{14}C-phenanthrene mineralisation, with higher maximum rates of mineralisation following 20 d aging. Higher extents of ^{14}C-phenanthrene mineralisation were observed in the Kettering loam soil (high OM), which was more prominent with 0.01% biochar amendments ($p < 0.05$) at 61.2% and 64.9% in ≤2 mm and 3–7 mm biochar amended soils, respectively. This study illustrates the potential role for biochar to enhance microbial catabolic activity to degrade common petroleum contaminants. It however depends on contaminant concentration, aging period, and soil properties.

Keywords: biochar; PAHs; catabolism; adaptation

1. Introduction

Soils contain a wide variety of indigenous microflora that possess catabolic potential to degrade contaminants, such as polycyclic aromatic hydrocarbons (PAHs) [1]. Indeed, microbial degradation is a major loss pathway for PAHs from soil [1,2]. The ability of microbes to adapt to PAHs and the time required for adaptation to occur, in part, determines the persistence of organic contaminants [3]. For example, Johnson and Karlson [4] showed that previous exposure to PAHs at levels greater than background levels influenced adaptation and the ability of the indigenous microbes to degrade PAHs. Furthermore, prolonged exposure to a contaminant can lead to greater catabolic activity of indigenous microbes [5,6]. However, the catabolic potential of microbes is influenced by contaminant concentration, bioavailability, chemical stability [7,8], and the presence of co-contaminants [9] and non-aqueous phase liquids (NAPLs) [10]. The adaptation period may further be affected by soil organic matter (SOM) which may provide a more readily available nutrient source than some aromatic contaminants, thus delaying the microbial need for contaminant breakdown [11].

Biochar (black carbon derived from pyrolysis of biomass) is a form of organic matter that is ubiquitous in nature [12]. Biochars retain some of the chemical properties of the original feedstock used in production, the extent of which depends on the conditions of pyrolysis [13]. Its presence in soil has been shown to influence mobility and bioavailability of organic contaminants as biochar can sequester such compounds reducing their mobility and degradability [14]. Biochar has been shown to adsorb organic compounds more strongly and nonlinearly to its carbonised phase than SOM [15,16]. The sorption mechanism of aromatic hydrocarbons, such as PAHs, onto wood char is assisted by π -electron interaction and pore-filling mechanism [17]. A fraction of PAHs may be sorbed to exterior surfaces of biochar and other portions are trapped within internal nanopores, thereby limiting mass transfer to microorganisms [18].

Recent studies have investigated the influence of the combined roles SOM and biochar amendments on the sorption of organic and inorganic contaminants in soils and sediments [15,19]. Biochar is a more important sorbent than SOM [20]. This suggests that SOM may compete for adsorption sites and block pore sites on black carbon. Furthermore, Rhodes *et al*. [1] showed that higher concentrations (>0.1%) of activated charcoal (AChar) reduced the extent and rate of ^{14}C-phenanthrene mineralisation in soils by reducing indigenous microbial catabolic activity. More recently, Bushnaf *et al*. [21] showed rapid biodegradation of alkanes in biochar amended soils. However, there is very limited information on the effect of different particle sizes of biochar on catabolic activity of indigenous microorganisms in soils varying in SOM content. Therefore, the aim of this current study is to investigate the role of different particle sizes and doses of wood-derived biochar on the catabolic activity of microorganisms in ^{14}C-phenanthrene spiked soils with differing SOM content. It further explores the effect of pre-exposure of ^{12}C-phenanthrene on microbial catabolic activity in biochar amended soils during aging.

2. Materials and Methods

2.1. Materials

Both non-labelled and [9-^{14}C] phenanthrene (98% radioactive purity) were obtained from Sigma-Aldrich, UK. Goldstar multipurpose scintillation cocktail was obtained from Meridian, UK. Nutrient agar and purified agar were obtained from Oxoid, UK. Biochar was provided by Yorkshire Charcoal Co., UK and was produced by slow pyrolysis (16–18 h duration at 450–500°C) of mixed wood feedstock, containing approximately 90% *Acer*, and the remaining 10% a mixture of *Quercus* and *Fraxinus* species. The biochar was separated to obtain two different ranges of particle sizes (≤2 mm and 3–7 mm) designated as BioC1 and BioC2, respectively.

2.2. Soil Preparation for Aging and Spiking

Two soils were used, chosen for their differing SOM contents. The physical properties of both soils are listed in Table 1. The low organic matter soil was obtained from a Myerscough pasture field at Myerscough Agricultural College, UK, and had a sandy loam texture. Kettering loam was the higher OM soil and was provided by Boughton Loam Ltd (Kettering, UK) as shown in Table 1 [22]. Soils were sieved through a 2 mm mesh to homogenise the soils and remove large debris such as plant roots and stones. Each of the soils were rehydrated with deionised water to their required moisture content and individually amended with biochar. The ≤2 mm (BioC1) and 3–7 mm (BioC2) biochar fractions were amended into the soils in triplicate in the following concentrations: 0%, 0.01%, 0.1% and 1%. To ensure homogeneity, samples were blended using stainless spoon for 8 min [1,23]. The soils were then spiked with non-labelled ^{12}C-phenanthrene standards prepared in toluene to achieve a concentration of 50 mg kg^{-1} as per Doick *et al*. [23]. The treatments were stored in separate amber jars in the dark at 21 ± 1 °C for the duration of aging (1, 20, 60 and 100 days). NMR-Cryoporometry analysis was performed to know total pore volume and liquid per unit mass of individual biochars as per Webber *et al*. [24]. Ash content was measured by heating biochar samples at 760 °C for 6 h [25] using a Carbolite Furnace RHF 1400 and calculated using the equation: Ash content (%) = (Bb − Ba/Bb) × 100, whereby Bb and Ba were biochar weight before and after heating, respectively [26]. Biochar pH analysis was measured in triplicate at 1% (d/w). The mixture was shaken for 24 h at 100 rpm and then measured using a digital pH meter. Results of biochar analysis are shown in Table 2.

Table 1. Physical properties of Myerscough (Clay-Loam) and Kettering Loam (Sandy-Loam). Data also available in Boucard *et al*. [22] and Couling *et al*. [9].

Soil Property	Myerscough	Kettering
pH	6.53	7.4
Moisture content (%)	21.07	30.0
Water holding capacity (%)	35.02	40.3
Clay (%)	19.50	23.0
Silt (%)	20.00	35.0
Sand (%)	60.40	42.0
Total organic matter (%)	2.70	5.0

Table 2. Biochar physical properties.

Temperature (°C)	Particle Size (mm)	pH	Ash Content (%)	Pore Volume (mL g^{-1})	Liquid Quantity (µL g^{-1})
450–500	≤2	9.6	13.7	1.39	44
450–500	3–7	9.6	14.4	2.20	60

2.3. Measuring ^{14}C-Phenanthrene Mineralisation by Indigenous Soil Microorganisms

After each aging period, soils were spiked with ^{12}C- and ^{14}C-phenanthrene (50 mg kg^{-1} at 41.67 Bq g^{-1} of soil) with toluene as the carrier solvent [27]. The mineralisation of ^{14}C-phenanthrene was determined by measuring the evolution of $^{14}CO_2$ using modified 250 mL Schott bottles containing 1 M NaOH (1 mL) as a CO_2 trap, which was replaced daily [27]. After 1, 20, 60 and 100 d aging in the dark at 21 ± 1 °C, respirometers were prepared in triplicate, with 10 ± 0.2 g soil (w/w) and 30 mL sterilized minimal basal salts (MBS) solution. MBS solution was made as follows: 0.3 g L^{-1} NaCl, 0.6 g L^{-1} $(NH_4)_2SO_4$, 0.6 g L^{-1} KNO_3, 0.25 g L^{-1} KH_2PO_4, 0.75 g L^{-1} K_2HPO_4, 0.15 g L^{-1} $MgSO_4 \cdot 7H_2O$, it also contained required micronutrients: 20 µg L^{-1} $LiCl(LiBO_2)$, 80 µg L^{-1} $CaSO_4 \cdot 5H_2O$, 100 µg L^{-1} $ZnSO_4 \cdot 7H_2O$, 100 µg L^{-1} $Al(SO_4)_3 \cdot 16H_2O$, 100 µg L^{-1} $NiCl \cdot 6H_2O(CoNO_3)$, 100 µg L^{-1} $CoSO_4 \cdot 7H_2O(CoNO_3)$, 30 µg L^{-1} KBr, 30 µg L^{-1} KI, 600 µg L^{-1} $MnCl_2 \cdot 2H_2O$, 40 µg L^{-1} $SnCl_2 \cdot 2H_2O$ and 300 µg L^{-1} $FeSO_4 \cdot 7H_2O$. Doick and Semple [28] recommend a 1:3 soil:liquid ratio for greater reproducibility and overall extents of mineralisation. The respirometers were then secured onto a SANYO Gallenkamp orbital shaker at 100 rpm, at 21 ± 1 °C in the dark for 14 days. At each sampling time (24 h) the trapped $^{14}CO_2$ was determined by adding 5 mL of Goldstar scintillation cocktail. These samples were counted using a Canberra Packard Tri-Carb 2250CA liquid scintillation analyser, utilising standard protocols and automatic quench correction.

2.4. Statistical Analyses

Statistical significances for overall extents of mineralisation, fastest rates of mineralisation and lag phases were evaluated using an analysis of variance (ANOVA). ANOVA ($p < 0.05$) was used to investigate significant differences amongst biochar amendments and particle sizes within each time points. Statistical analysis was done using SigmaStat software (Version 2.0).

3. Results

3.1. Effects of Biochar Amendment on Mineralisation of ^{14}C-Phenanthrene

The mineralisation of ^{14}C-phenanthrene to $^{14}CO_2$ over a 14 d experimental period was measured in both the Kettering loam and Myerscough soils amended with 0%, 0.01%, 0.1% and 1% biochar (BioC1 & BioC2), after 1, 20, 60 and 100 d soil-phenanthrene aging period. The effect of each biochar focused on changes in the lag phase, rate and extent of ^{14}C-phenanthrene mineralisation in the soils following pre-exposure to ^{12}C-phenanthrene.

3.1.1. Impact of Biochar on the Lag Phase Prior to Mineralisation of ^{14}C-Phenanthrene in Soil

Lag phases were defined and measured as the time taken for the extent of mineralisation of ^{14}C-phenanthrene to exceed 5% of the amount applied in each respirometry bottle. Differences in soils showed that lag phase was greater in the Myerscough soil compared to the Kettering loam (Tables 3 and 4) and was further evident in biochar amended soils. For instance at 1 d incubation, 0.01%, 0.1% and 1% BioC2 amendments in the Myerscough soil showed statistically greater ($p < 0.01$) lag phases than 0.01%, 0.1% and 1% BioC2 in the Kettering loam, the lag phases lasted between 4 and 9 d for the Myerscough soil and remained at approximately 3 d for Kettering loam soil (Tables 3 and 4). When particles sizes were compared over time, there was statistically insignificant difference ($p > 0.05$) in the lag phases. Following 20 d aging period in both soils (Myerscough and Kettering loam), there was very little real difference in lag phases but these were shown to decline significantly ($p < 0.05$) to a maximum of 2.9 and 2.7 d, respectively. Noticeably, the 20 d soil-phenanthrene aging period exhibited the shortest lag phases, irrespective of biochar concentration (Tables 3 and 4; Figures 1 and 2). The reduction in lag phase was not consistent with an increase in aging period, rather, a minimum of 30% increase in lag phase was observed following 60 and 100 d time point compared to 20 d time point. The addition of biochar to the soils had an effect after 20 d, where increasing concentrations of biochar led to a decreasing lag phase in the Myerscough soil and an increased lag phase in the Kettering loam after 100 d aging period (Tables 3 and 4), when compared to controls.

Table 3. Mineralisation of ^{14}C-phenanthrene in Myerscough and Kettering loam soils amended with 0%, 0.01%, 0.1% and 1% of ≤ 2 mm biochar (BioC1). Errors represent standard error of mean (SEM) of triplicate samples ($n = 3$).

Aging Period (d)	Soil	Amendment (%)	Lag Phase (d)	Maximum Rates (% d^{-1})	Total Extent (%)
1	Myerscough	0	7.09 ± 1.86 ^{aA}	17.69 ± 0.98 ^{aA}	42.59 ± 4.30 ^{aA}
		0.01	7.80 ± 1.29 ^{aA}	25.09 ± 3.03 ^{aA}	45.07 ± 0.03 ^{aB}
		0.1	5.48 ± 0.87 ^{aA}	26.04 ± 3.10 ^{aA}	48.44 ± 1.47 ^{aB}
		1	4.94 ± 0.64 ^{aA}	29.67 ± 3.13 ^{aA}	34.34 ± 2.62 ^{aB}
	Kettering	0	3.31 ± 0.10 ^{aB}	20.00 ± 1.95 ^{aA}	45.39 ± 1.28 ^{bA}
		0.01	3.15 ± 0.03 ^{aB}	32.73 ± 4.08 ^{aA}	61.16 ± 0.89 ^{aA}
		0.1	3.13 ± 0.03 ^{aA}	28.62 ± 3.79 ^{aA}	61.86 ± 1.57 ^{aA}
		1	3.18 ± 0.04 ^{aA}	26.76 ± 3.72 ^{aA}	58.58 ± 2.43 ^{aA}
20	Myerscough	0	2.93 ± 0.08 ^{aA}	15.29 ± 0.62 ^{aA}	49.08 ± 0.70 ^{aB}
		0.01	2.69 ± 0.09 ^{aA}	20.33 ± 1.80 ^{aA}	53.31 ± 0.80 ^{aA}
		0.1	2.21 ± 0.07 ^{bB}	21.77 ± 1.50 ^{aA}	53.58 ± 1.59 ^{aA}
		1	2.36 ± 0.06 ^{bB}	21.68 ± 0.63 ^{aA}	50.40 ± 2.01 ^{aB}
	Kettering	0	2.03 ± 0.01 ^{aB}	19.15 ± 0.73 ^{aA}	58.92 ± 0.90 ^{aA}
		0.01	2.08 ± 0.06 ^{aB}	16.87 ± 2.66 ^{aA}	57.45 ± 0.80 ^{aA}
		0.1	2.10 ± 0.02 ^{aA}	16.28 ± 1.92 ^{aA}	57.64 ± 1.68 ^{aA}
		1	2.66 ± 0.41 ^{aA}	13.65 ± 0.92 ^{aB}	58.14 ± 0.71 ^{aA}
60	Myerscough	0	3.56 ± 0.10 ^{aA}	16.40 ± 2.10 ^{bA}	43.69 ± 1.36 ^{aA}
		0.01	3.83 ± 0.16 ^{aA}	29.50 ± 3.09 ^{aA}	56.08 ± 1.26 ^{aA}
		0.1	2.96 ± 0.07 ^{aA}	29.02 ± 2.72 ^{aA}	50.15 ± 1.70 ^{aA}
		1	3.18 ± 0.07 ^{aA}	21.61 ± 1.87 ^{bA}	52.16 ± 2.28 ^{aA}

Table 3. *Cont.*

Aging Period (d)	Soil	Amendment (%)	Lag Phase (d)	Maximum Rates (% d^{-1})	Total Extent (%)
60	Kettering	0	2.62 ± 0.05 ^{aB}	10.09 ± 0.39 ^{aA}	34.46 ± 3.21 ^{aA}
		0.01	2.52 ± 0.25 ^{aA}	15.26 ± 1.72 ^{aB}	42.49 ± 0.72 ^{aB}
		0.1	2.50 ± 0.28 ^{aA}	12.27 ± 1.62 ^{aB}	43.85 ± 1.03 ^{aA}
		1	2.48 ± 0.04 ^{aB}	10.66 ± 0.30 ^{aB}	41.23 ± 1.87 ^{aB}
100	Myerscough	0	3.92 ± 0.02 ^{aA}	12.79 ± 1.46 ^{aA}	36.26 ± 1.40 ^{aA}
		0.01	4.12 ± 0.07 ^{aA}	12.79 ± 1.36 ^{aA}	37.29 ± 1.07 ^{aA}
		0.1	3.78 ± 0.15 ^{aA}	17.78 ± 1.03 ^{aA}	44.08 ± 1.52 ^{aA}
		1	3.46 ± 0.04 ^{aA}	10.23 ± 1.01 ^{aA}	37.29 ± 0.83 ^{aA}
	Kettering	0	2.38 ± 0.06 ^{aB}	13.70 ± 1.27 ^{aA}	44.07 ± 1.08 ^{aA}
		0.01	2.26 ± 0.01 ^{aB}	17.02 ± 1.23 ^{aA}	47.02 ± 2.46 ^{aA}
		0.1	2.41 ± 0.04 ^{aB}	15.55 ± 3.14 ^{aA}	42.26 ± 2.99 ^{aA}
		1	2.51 ± 0.07 ^{aB}	10.76 ± 2.63 ^{aA}	41.03 ± 1.71 ^{aA}

^{a}: No statistical significant difference ($p > 0.05$) in soils amended with biochar compared to control; ^{A}: No statistical significant difference ($p > 0.05$) in same biochar dose but different soil types; ^{b}: Statistical significant difference ($p < 0.05$) in soils amended with biochar compared to control; ^{B}: Statistical significant difference ($p < 0.05$) in same biochar dose but different soil types.

Table 4. Mineralisation of ^{14}C-phenanthrene in Myerscough and Kettering loam soils amended with 0%, 0.01%, 0.1% and 1% of 3–7 mm biochar. Errors represent standard error of mean (SEM) of triplicate samples ($n = 3$).

Aging Period (d)	Soil	Amendment (%)	Lag Phase (d)	Maximum Rates (% d^{-1})	Total Extent (%)
1	Myerscough	0	7.09 ± 1.86 ^{aA}	17.69 ± 0.98 ^{aA}	42.59 ± 4.30 ^{aA}
		0.01	7.01 ± 0.70 ^{aA}	18.95 ± 2.87 ^{aB}	42.45 ± 0.50 ^{aB}
		0.1	8.66 ± 0.10 ^{aA}	17.95 ± 0.91 ^{aB}	41.39 ± 0.20 ^{aB}
		1	7.08 ± 0.02 ^{aA}	15.49 ± 2.20 ^{aB}	36.21 ± 3.00 ^{aB}
	Kettering	0	3.31 ± 0.10 ^{aB}	20.00± 1.95 ^{bA}	45.39 ± 1.28 ^{bA}
		0.01	3.27 ± 0.17 ^{aB}	30.75 ± 2.98 ^{aA}	64.92 ± 1.07 ^{aA}
		0.1	3.18 ± 0.06 ^{aB}	26.64 ± 0.70 ^{aA}	53.76 ± 2.06 ^{aA}
		1	3.35 ± 0.55 ^{aB}	14.72 ± 1.68 ^{bA}	50.32 ± 1.20 ^{bA}
20	Myerscough	0	2.93 ± 0.08 ^{aA}	15.29 ± 0.62 ^{aA}	49.08 ± 0.70 ^{bB}
		0.01	2.43 ± 0.09 ^{bA}	24.10 ± 2.39 ^{aA}	52.19 ± 1.08 ^{aB}
		0.1	2.24 ± 0.11 ^{bA}	23.84 ± 1.22 ^{aA}	55.17 ± 0.61 ^{aA}
		1	2.38 ± 0.12 ^{bA}	22.21 ± 1.82 ^{aA}	53.84 ± 0.19 ^{aA}
	Kettering	0	2.03 ± 0.08 ^{bB}	19.15 ± 0.73 ^{aA}	58.92 ± 0.90 ^{aA}
		0.01	2.12 ± 0.01 ^{aB}	17.62 ± 0.04 ^{aB}	56.48 ± 0.88 ^{aA}
		0.1	2.09 ± 0.03 ^{aA}	15.89 ± 1.11 ^{aB}	57.83 ± 0.79 ^{aA}
		1	2.07 ± 0.01 ^{aB}	13.39 ± 1.07 ^{aB}	55.86 ± 0.78 ^{aA}
60	Myerscough	0	3.56 ± 0.10 ^{aA}	16.40 ± 2.10 ^{bA}	43.69 ± 1.36 ^{aA}
		0.01	3.13 ± 0.02 ^{aA}	22.23 ± 0.22 ^{aA}	49.20 ± 0.87 ^{aA}
		0.1	2.54 ± 0.20 ^{bA}	24.76 ± 1.91 ^{aA}	52.26 ± 2.28 ^{aA}
		1	2.61 ± 0.16 ^{bA}	24.46 ± 1.82 ^{aA}	51.82 ± 0.73 ^{aA}

Table 4. *Cont.*

Aging Period (d)	Soil	Amendment (%)	Lag Phase (d)	Maximum Rates (% d^{-1})	Total Extent (%)
60	Kettering	0	2.62 ± 0.05 ^{aB}	10.09 ± 0.39 ^{bA}	34.46 ± 3.21 ^{aA}
		0.01	2.40 ± 0.07 ^{bB}	9.72 ± 0.55 ^{bB}	40.82 ± 1.31 ^{aB}
		0.1	2.30 ± 0.05 ^{bA}	13.90 ± 0.80 ^{aB}	43.07 ± 1.85 ^{aB}
		1	2.34 ± 0.03 ^{bA}	13.50 ± 0.62 ^{aB}	42.23 ± 1.71 ^{aB}
100	Myerscough	0	3.92 ± 0.02 ^{aA}	12.79 ± 1.46 ^{aA}	36.26 ± 1.40 ^{aA}
		0.01	3.90 ± 0.10 ^{aA}	9.75 ± 1.21 ^{aA}	38.69 ± 3.37 ^{aA}
		0.1	3.63 ± 0.03 ^{bA}	12.79 ± 1.46 ^{aB}	36.70 ± 2.45 ^{aB}
		1	3.25 ± 0.02 ^{bA}	14.66 ± 2.15 ^{aA}	43.61 ± 0.95 ^{aA}
	Kettering	0	2.38 ± 0.06 ^{aB}	13.70 ± 1.27 ^{aA}	44.07 ± 1.08 ^{aA}
		0.01	2.62 ± 0.18 ^{aB}	14.96 ± 3.51 ^{aA}	42.40 ± 2.22 ^{aA}
		0.1	2.57 ± 0.11 ^{aB}	20.33 ± 0.37 ^{aA}	47.66 ± 0.61 ^{aA}
		1	2.55 ± 0.09 ^{aB}	15.81 ± 1.49 ^{aA}	44.60 ± 1.99 ^{aA}

^{a}: No statistical significant difference ($p > 0.05$) in soils amended with biochar compared to control; ^{A}: No statistical significant difference ($p > 0.05$) in same biochar dose but different soil types; ^{b}: Statistical significant difference ($p < 0.05$) in soils amended with biochar compared to control; ^{B}: Statistical significant difference ($p < 0.05$) in same biochar dose but different soil types.

3.1.2. *Impact of Biochar on the Maximum Rates of ^{14}C-Phenanthrene Mineralisation in Soil*

The mean maximum rates of mineralisation over the 14 d experimental period for each biochar amendment in both soils at all soil-phenanthrene aging periods was calculated (Tables 3 and 4). The maximum rates of mineralisation were attained within 3–10 days and ≤3 days of mineralisation for the Myerscough and Kettering loam soils, respectively. After 1 d soil-phenanthrene aging period, there was generally insignificant difference ($p > 0.05$) in the maximum rate of $^{14}CO_2$ evolution per day in both soils and particle size amendments. However, the presence of biochar during soil-phenanthrene aging maintained maximum rates of mineralisation in both soils until there was increase in aging where biochar increased maximum rates in both soils following 20 and 60 d soil-phenanthrene aging period (Tables 3 and 4). For instance, the highest rate of mineralisation (30%–33% d^{-1}) was observed in 0.01% of both particle sizes of biochar-amended to Kettering loam. Afterwards (100 d), rates of mineralisation decreased ($p < 0.05$) to be consistent with those in the control soil (Tables 3 and 4).

3.1.3. Impact of Biochar on the Total Extents of ^{14}C-Phenanthrene Mineralisation

The extent of $^{14}CO_2$ production over 14 d varied in both soils, with increasing soil-phenanthrene aging period (Figures 1 and 2; Tables 3 and 4). When the extent of mineralisation was compared amongst the different soil-phenanthrene aging periods (1, 20, 60 and 100 d), there were statistical differences. The extent of mineralisation did not exceed 50% of the dose applied to the controls; however, the addition of both biochars (BioC1 and BioC2) resulted in statistical increases ($p < 0.05$) in the extent of ^{14}C-phenanthrene mineralisation in Kettering loam soil after 1 d soil-phenanthrene aging period. However, this was not consistent with increasing soil aging. Although 1% biochar amendment of both particle sizes (≤2 mm and 3–7 mm) resulted in a lower extent of ^{14}C-phenanthrene

mineralisation when compared to the control soil; this was not statistically significant ($p > 0.05$) (Table 3). Following an increase in soil-phenanthrene aging period, biochar maintained the extent of ^{14}C-phenanthrene mineralisation in both soils in comparison to the control soil, with no further statistical increase ($p > 0.05$). For example, the extent of mineralisation in the biochar-amended Kettering loam soil ranged between 55%–59%, 40%–44% and 41%–48% after 20, 60 and 100 d aging, respectively, whilst in the Myerscough soil, it ranged between; 50%–55%, 49%–56% and 36%–44% after 20, 60 and 100 d aging, respectively. There were, however insignificant differences ($p > 0.05$) between the two particle sizes on the extent of ^{14}C-phenanthrene mineralisation in both soils. In the Myerscough soil, there was a minimum of 7% increase in the extent of mineralisation after 20 d aging compared to 1 d aging. Following further aging (60 and 100 d), the extent of mineralisation after 20 d aging were statistically greater ($p < 0.05$) than 100 d aging irrespective of biochar concentration and particle size (Table 4; Figure 1). In contrast, biochar amendments in Kettering loam showed that extents of mineralisation after 20 d aging was statistically greater ($p < 0.05$) than mineralisation after 60 and 100 d aging, but similar to 1 d aging (Table 3; Figure 2). Despite an increase in the extent of mineralisation after 20 d soil aging in the Kettering loam, it showed a statistically greater ($p < 0.05$) extent of mineralisation when amended with biochar than the Myerscough soil at 1 and 100 d soil aging.

4. Discussion

The microbial communities within both soils were able to mineralise the ^{14}C-phenanthrene over 14 days at each time point (1, 20, 60 and 100 d). However, this was not achieved without the adaptation of the indigenous microorganisms within each soil. All the curves of mineralisation in this study were sigmoidal (Figures 1 and 2). Suggesting that the phenanthrene-degrading populations were continually growing [5,9]. The adaptation was probably through one or more mechanisms, such as an increase in microbial population, induction of catabolic enzymes, transgenic manipulations via horizontal gene transfer within the degrading populations [29]. After 1 d soil-phenanthrene aging, biochar amendments had no impact on the lag phase in both Myerscough and Kettering loam soils. It has been suggested that longer initial adaptation periods results in shorter lag phases and greater rates of mineralisation [5]. However, the shorter lag phases experienced in the Kettering loam soil compared to the Myerscough soil was apparently due to the difference in soil properties and indigenous microflora [1,30]. After 20 d aging of ^{12}C-phenanthrene, both the Myerscough and Kettering loam soils experienced significant decreases in lag phases which support Rhodes *et al*. [1,30], where lag phases decreased after 42 and 25 d aging periods, respectively. Such increase in soil-phenanthrene contact time allowed continued proliferation of microbial degrading organisms that eventually enhanced degradation of the phenanthrene bioavailable fraction [1,4]. Despite decreases in the lag phase in this current study, indigenous soil populations never fully acclimatised to the addition of phenanthrene since the sigmoidal shape was always apparent (Figures 1 and 2). To explain this, the time taken to mineralise a particular PAH depends on level of contamination in soil and soil type [1,4]. With respect to concentration effects, higher concentrations of PAHs can be toxic to degraders and inhibit their growth [9], also, 10 mg kg^{-1} of phenanthrene was spiked into soils by Rhodes *et al*. [1,30], whilst 50 mg kg^{-1} of phenanthrene was spiked in soils in this current study. After 20 d aging, biochar amendments (0.1% and 1%) decreased the lag phase in the Myerscough soil to a greater extent compared to the control soil.

Biochar has the potential to interact with the soil matrix and adsorb growth-inhibiting substances [31]. Furthermore, the presence of biochar alters nutrient availability for microbial utilisation within soils following aging [32,33]. In the Kettering loam soil, it took 20 days of soil-phenanthrene aging to reduce the lag phase from 3.3 d to 2 d. This means that after pre-exposure of phenanthrene to indigenous microorganisms in biochar amended soils, the lag phase will be decreased depending on soil-phenanthrene aging period, and soil characteristics.

Figure 1. Mineralisation of ^{14}C-phenanthrene in Myerscough soil amended with biochar at 0% (○), 0.01% (▽), 0.1% (□) and 1% (◊) after 1, 20, 60 and 100 d soil-phenanthrene aging periods. Error bars represent standard error of mineralisation (SEM) (n = 3).

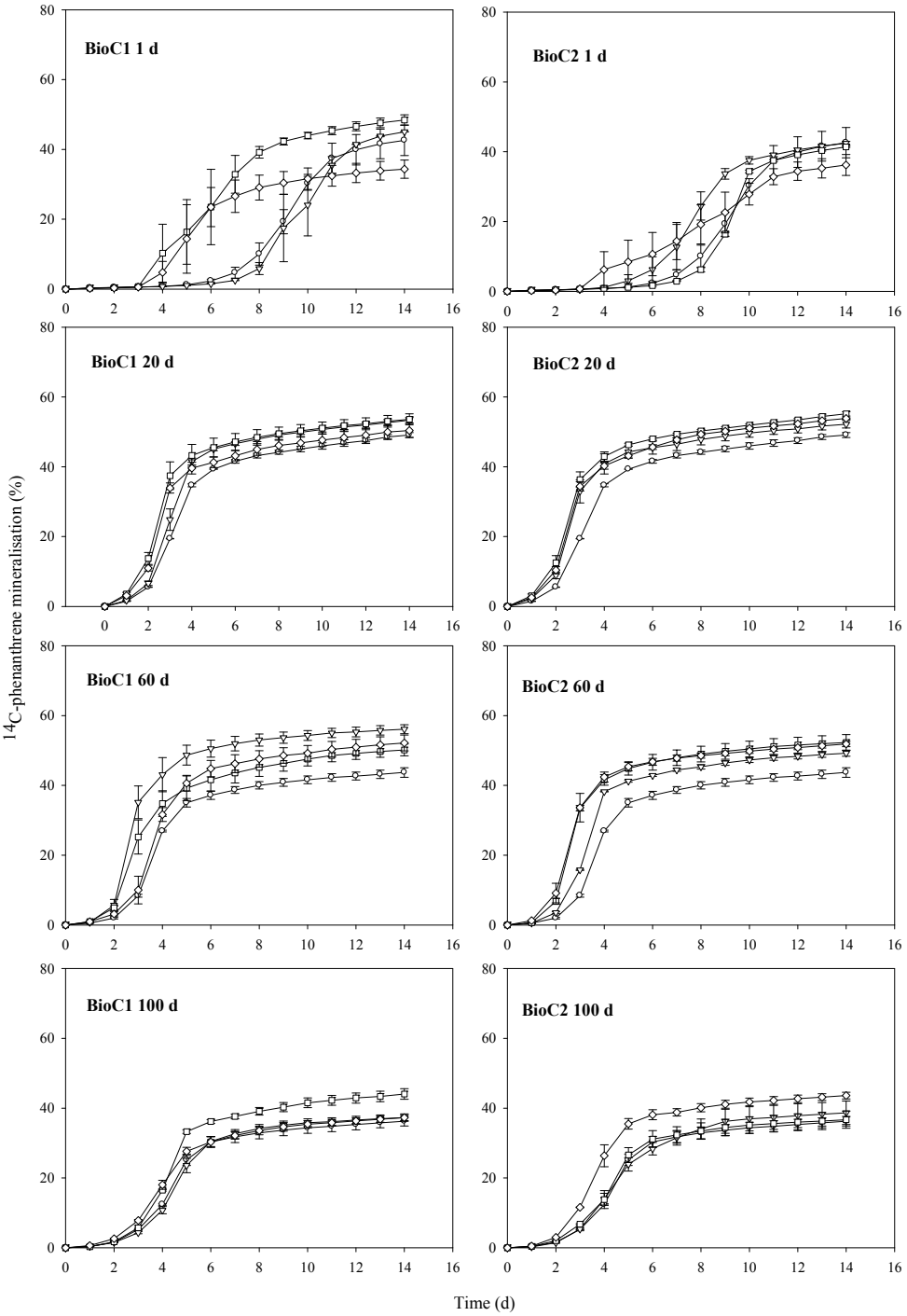
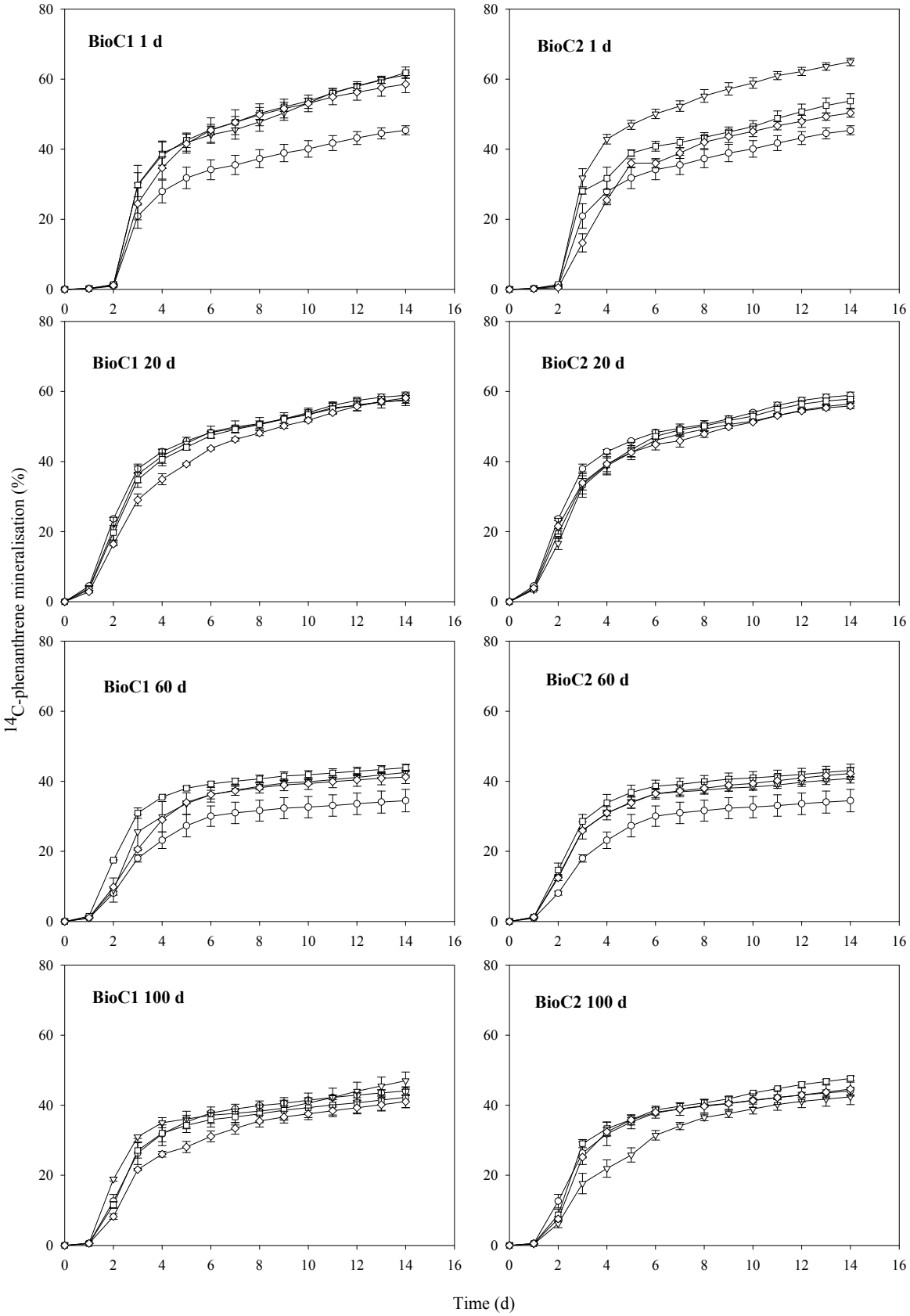


Figure 2. Mineralisation of ^{14}C-phenanthrene in Kettering loam soil amended with biochar at 0% (○), 0.01% (▽), 0.1% (□) and 1% (◊) after 1, 20, 60 and 100 d soil-phenanthrene aging periods. Error bars represent standard error of mineralisation (SEM) (n = 3).



Maximum rates of mineralisation showed neither significant differences between soils nor between the control and biochar amended samples at 1 d soil-phenanthrene aging, which concurs with the lag phase results. The fastest rates of $^{14}CO_2$ evolution commonly occur during the exponential phase of mineralisation (Tables 3 and 4; Figures 1 and 2). In this study, the addition of biochar of both particle sizes (≤2 mm and 3–7 mm) produced higher maximum rates of mineralisation in both the Myerscough and Kettering loam soils than in the control following increased soil-phenanthrene aging. Similarly, Bushnaf *et al*. [21] showed that biodegradation of water-dissolved and available gasoline constituents were faster in 2% biochar-amended soils than in unamended soils. Kolb *et al*. [34] equally demonstrated that the addition of manure/wood biochar (500 °C) to 4 different soil types expressed an increasing microbial activity at 1, 2.5, 5 and 10% charcoal amendment, but depended on soil type. In addition, increasing concentrations of biochar in Walla Walla loam soils resulted in increased microbial activity and respiration in first few days of incubation, and then decreased after 6 d [35]. Although this is contrary to Rhodes *et al*. [1], where 1% AChar resulted in reduction in maximum rates of mineralisation and created long lag phases. This difference is apparently due to the difference in black carbon material used, phenanthrene concentration and soil properties. The biochar used in this study was not subject to any form of chemical activation to increase microporosity. Also, biochar is a preferred habitat for microbial growth than some activated carbon [36] and enhances microbial catabolic ability in soils [37,38]. Similarly, the availability of C, N and other nutrients from biochar amended soils increases microbial colonisation, ATP production and microbial production [39,40]. The biochar used in this study shows that amongst its pores, it contains more macropores (>50 nm) that encourage microbial colonisation and reproduction [41] (Table 2). Additionally, the biochars also contain liquid hydrocarbons (Table 2) within the pores that can serve as substrates for growth and metabolism [42]. Despite presence of OM in soils, the degree of competition for sorption site between contaminant and OM felt by the adsorbate will decrease from low to high concentrations [43]. It thus means that biochar does not discourage catabolic activity in soils.

At 1 d soil-phenanthrene aging, the two soil types exhibited contrary responses to the effect of biochar amendment on total extents of ^{14}C-phenanthrene mineralisation. Higher extents of mineralisation in 0.01% and 0.1% biochar-amended Kettering loam soil showed that low concentrations of wood biochar amendment can influence extent of phenanthrene biodegradation due to potential role of humic substances on biochar sorption sites and surface properties [19,43–45]. The biochar dose that led to response was low and a proper homogenous mixture with the soil would not be certain. Therefore there would have been some biochar hotspots within the soil-biochar mixture. However it is worthy to note that ^{14}C-phenanthrene was spiked directly into aqueous solution of the respirometer assay and not into soil. Furthermore, the slurry method used enables agitation which tends to aid distribution of microorganisms evenly [4,27], and facilitates degradation of phenanthrene, while soil particles separate into suspension [27]. In so doing, microorganisms adhere to BC particles to reduce distance to substrate and mineralise phenanthrene [9,46,47], whilst presence of microbes on pore sites and native SOM may attenuate sorption capacity of biochar [20]. Although not investigated, attachment of microorganisms to biochar is often through one of the following processes: (1) flocculation; (2) adsorption; (3) covalent bonding; (4) cross-linking of cells; (5) encapsulation in a polymer-gel; and (6) entrapment within matrix [31]. Zhou *et al*. [48] illustrated that modification with humic acids changes charcoal properties, which will eventually affect PAH desorption dynamics. PAH

concentration is a determining factor governing the extent of mineralisation in soils when investigating microbial catabolic activities in slurry method. This study supports Couling *et al*. [9], where greatest extents of ^{14}C-phenanthrene mineralisation did not exceed 60% at higher concentrations, despite aging in unamended biochar soils. A decline in microbial activity caused the lower extent of mineralisation due to toxicity of the higher concentration of phenanthrene.

5. Conclusions

This study shows soils contain an intrinsic microbial catabolic ability to degrade PAHs over time, but that this differs with soil characteristics and soil-PAH exposure time. The ability to degrade phenanthrene (a known PAH) was significantly enhanced (albeit to a minor extent) due to amendment of wood-derived biochar in soils [37,39]. This was expressed by shorter lag phases and higher maximum rates of ^{14}C-phenanthrene mineralisation in both soils, following increased soil-phenanthrene aging period. The findings also showed that the presence of low concentrations of such biochar does not inhibit the catabolic activity of indigenous microorganisms. It, however, depends on contaminant concentration, soil-contaminant pre-exposure time, and soil properties. Biochar retains much of the original chemical properties and features of the feedstock [13], which sustains PAH-degrading microorganisms. However, at higher concentrations of biochar, it is assumed there will be more available pore sites that will cause further sequestration of contaminants. This shows that biochar could be applied as a tool in the bioremediation process of contaminated land. Further studies will investigate the effect of biochar on the biodegradation of aged ^{14}C-PAHs in soils.

Acknowledgments

The authors are thankful to Petroleum Development and Technology Fund for partial funding. The biochar analysis (pore size distribution) was done by Beau Webber of Lab-Tools Ltd., Kent, UK. Additionally, we thank Aller Fernandez for her comments and advice in this research.

Author Contributions

Uchenna Ogbonnaya and Ayodeji Oyelami developed the initial idea of the research and designed the whole experiment. Justin Matthews was involved in the experimental sampling stage, data collection and initial presentation of data. Uchenna Ogbonnaya was responsible for following up on study participant and data cleaning. Kirk Semple and Olusoji Adebisi provided technical advice on the research. Uchenna Ogbonnaya drafted the manuscript and co-authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Rhodes, A.H.; McAllister, L.E.; Chen, R.; Semple, K.T. Impact of activated charcoal on the mineralisation of ^{14}C-phenanthrene in soils. *Chemosphere* **2010**, *79*, 463–469.

2. Semple, K.T.; Morriss, A.W.J.; Paton, G.I. Bioavailability of hydrophobic organic contaminants in soils: Fundamental concepts and techniques for analysis. *Eur. J. Soil Sci.* **2003**, *564*, 1–10.
3. Aeolin, C.M.; Bradley, D.C.; Pfaender, F.K. Adaptation of aquifer microbial communities to the biodegradation of xenobiotic compounds: Influence of substrate concentration and pre-exposure. *Environ. Tox. Chem.* **1989**, *8*, 75–86.
4. Johnson, A.R.; Karlson, U. PAH degradation capacity of soil microbial communities—Does it depend on PAH exposure? *Microbial. Ecol.* **2005**, *50*, 488–495.
5. Macleod, C.J.; Semple, K.T. The adaptation of two similar soils to pyrene catabolism. *Environ. Pollut.* **2002**, *119*, 357–364.
6. Brandt, B.W.; Kelpin, F.D.; van Leeuwen, I.M.; Kooijman, S.A. Modelling microbial adaptation to changing availability of substrates. *Water Res.* **2004**, *38*, 1003–1013.
7. Tian, Y.; Zheng, T.; Wang, X. PAHs contamination and PAH-degrading bacteria in Xiamen Western Sea. *Chem. Speciat. Bioavailab.* **2002**, *14*, 25–33.
8. Semple, K.T.; Doick, K.J.; Wick, L.Y.; Harms, H. Microbial interactions with organic contaminants in soil: Definitions, processes and measurements. *Environ. Pollut.* **2007**, *150*, 166–176.
9. Couling, N.R.; Towell, M.G.; Semple, K.T. Reprinted from Biodegradation of PAHs in soil: Influence of chemical structure, concentration and multiple amendment. *Environ. Pollut.* **2010**, *158*, 3411–3420.
10. Lee, P.H.; Doik, K.J.; Semple, K.T. The development of phenanthrene catabolism in soil amended with transformer oil. *FEMS Microbiol. Lett.* **2003**, *228*, 217–223.
11. Gourlay, C.; Tusseau-Vuillemin, M.H.; Mouchel, J.M.; Garric, J. The ability of dissolved organic matter (DOM) to influence benzo[a]pyrene bioavailability increases with DOM biodegradation. *Ecotoxicol. Environ. Saf.* **2005**, *61*, 74–82.
12. Braida, W.J.; Pignatello, J.J.; Lu, Y.; Ravikovitch, P.I.; Neimark, A.V.; Xing, B. Sorption hysteresis of benzene in charcoal particles. *Environ. Sci. Technol.* **2003**, *37*, 409–417.
13. Song, J.; Peng, P. Characterisation of black carbon materials by pyrolysis-gas chromatography-mass spectrometry. *J. Anal. Appl. Pyrolysis* **2010**, *87*, 129–137.
14. Beesley, L.; Moreno-Jiménez, E.; Gomez-Eyles, J.L. Effect of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. *Environ. Pollut.* **2010**, *158*, 2282–2287.
15. Accardi-Dey, A.; Gschwend, P.M. Assessing the combined roles of natural organic matter and black carbon as sorbents in sediments. *Environ. Sci. Technol.* **2002**, *3*, 21–29.
16. Cao, X.; Ma, L.; Gao, B.; Harris, W. Dairy-manure derived biochar effectively sorbs lead and atrazine. *Environ. Sci. Technol.* **2009**, *43*, 3285–3291.
17. Chen, B.; Chen, Z. Sorption of naphthalene and 1-naphthol by biochar of orange peels with different pyrolytic temperatures. *Chemosphere* **2009**, *76*, 127–133.
18. Huang, W.; Peng, P.; Yu, Z.; Fu, J. Effects of organic matter heterogeneity on sorption and desorption of organic contaminants by soils and sediments. *Appl. Geochem.* **2003**, *18*, 955–972.
19. Kasozi, G.N.; Zimmerman, A.R.; Nkedi-Kizza, P.; Gao, B. Catechol and humic acid sorption onto a range of laboratory produced black carbons (biochars). *Environ. Sci. Technol.* **2010**, *44*, 6189–6195.

20. Cornelissen, G.; Gustafsson, O. Sorption of phenanthrene to environmental black carbon in sediment with and without organic matter and native sorbates. *Environ. Sci. Technol.* **2004**, *38*, 148–155.
21. Bushnaf, K.M.; Puricelli, S.; Saponaro, S.; Werner, D. Effect of biochar on the fate of volatile petroleum hydrocarbons in an aerobic sandy soil. *J. Contam. Hydrol.* **2011**, *126*, 208–215.
22. Boucard, T.K.; Bardgett, R.D.; Jones, K.C.; Semple, K.T. Reprinted from influence of plants on the chemical extractability and biodegradability of 2,4-dichlorophenol in soil. *Environ. Pollut.* **2005**, *133*, 53–62.
23. Doick, K.J.; Lee, P.H.; Semple, K.T. Assessment of spiking procedures for the introduction of a phenanthrene-LNAPL mixture into a field-wet soil. *Environ. Pollut.* **2003**, *126*, 399–406.
24. Webber, J.B.W.; Corbett, P.; Semple, K.T.; Ogbonnaya, U.; Teel, W.S.; Masiello, C.A.; Fisher, Q.J.; Valenza, J.J., II; Song, Y.Q.; Hu, Q. An NMR study of porous rock and biochar containing organic material. *Micropor. Mesopor. Mat.* **2013**, *178*, 94–98.
25. Novak, J.M.; Lima, I.; Xing, B.; Gaskin, J.W.; Steiner, C.; Das, K.C.; Ahmedna, M.; Rehrah, D.; Watts, D.W.; Busscher, W.J.; *et al*. Characterization of designer biochar produced at different temperatures and their effects on loamy sand. *Ann. Environ. Sci.* **2009**, *3*, 195–206.
26. Cao, X.; Harris, W. Properties of dairy-manure derived biochar pertinent to its potential use in remediation. *Bioresour. Technol.* **2010**, *100*, 5222–5228.
27. Reid, B.J.; MacLeod, C.J.A.; Lee, P.H.; Morriss, A.W.J.; Stokes, J.D.; Semple, K.T. A simple ^{14}C-respirometric method for assessing microbial catabolic potential and contaminant bioavailability. *FEMS Microbiol. Lett.* **2001**, *196*, 141–146.
28. Doick, K.J.; Semple, K.T. The effect of soil: Water ratios on the mineralization of phenanthrene: LNAPL mixtures in soil. *FEMS Microbiol. Lett.* **2003**, *220*, 29–33.
29. Top, E.M.; Springael, D. The role of mobile genetic elements in bacterial adaptation to xenobiotic organic compounds. *Curr. Opin. Biotechnol.* **2003**, *14*, 262–269.
30. Rhodes, A.H.; Hofman, J.; Semple, K.T. Development of phenanthrene catabolism in natural and artificial soils. *Environ. Pollut.* **2008**, *152*, 424–430.
31. Lehmann, J.; Rillig, M.C.; Thies, J.; Masiello, C.A.; Hockaday, W.C.; Crowley, D. Biochar effects on soil biota—A review. *Soil Biol. Biochem.* **2011**, *43*, 1812–1836.
32. Glaser, B.; Lehmann, J.; Zech, W. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—A review. *Biol. Fertil. Soils* **2002**, *35*, 219–230.
33. Gundale, M.J.; Deluca, T.H. Temperate and source material influence ecological attributes of ponderosa pine and Douglas-fir charcoal. *For. Ecol. Manag.* **2006**, *231*, 86–93.
34. Kolb, S.E.; Fermanich, K.J.; Dornbush, M.E. Effect of charcoal quantity on microbial biomass and activity in temperate soils. *Soil Sci. Soc. Am. J.* **2009**, *73*, 1173–1181.
35. Smith, J.L.; Collins, H.P.; Bailey, V.L. The effect of young biochar on soil respiration. *Soil Biol. Biochem.* **2010**, *42*, 2345–2347.
36. Pietikäinen, J.; Kiikkila, O.; Fritze, H. Charcoal as a habitat for microbes and its effects on microbial community of the underlying humus. *Oikos* **2000**, *89*, 231–242.
37. Demeyer, A.; Voundi Nkana, J.C.; Verloo, M.G. Characteristics of wood ash and influence on soil properties and nutrient uptake: An overview. *Bioresour. Technol.* **2001**, *77*, 287–295.

38. Steinbeiss, S.; Gleixner, G.; Antonietti, M. Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biol. Biochem.* **2009**, *41*, 1301–1310.
39. Kogel-Knabner, I. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biol. Biochem.* **2002**, *34*, 139–162.
40. Luo, Y.; Durenkamp, M.; de Nobili, M.; Lin, Q.; Devonshire, B.J.; Brookes, P.C. Microbial biomass growth, following incorporation of biochars produced at 350 °C or 700 °C, in a silty-clay loam soil of high and low pH. *Soil Biol. Biochem.* **2013**, *57*, 513–523.
41. Thies, J.E.; Rillig, M.C. Characteristics of Biochar: Biological Properties. In *Biochar for Environmental Management*; Lehmann, J., Joseph, S., Eds.; Earthscan: Sterling, VA., USA, 2009; pp. 85–105.
42. Steiner, C.; Das, K.C.; Garcia, M.; Forster, B.; Zech, W. Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. *Pedobiologia* **2008**, *51*, 359–366.
43. Pignatello, J.J.; Kwon, S.; Lu, Y. Effect of natural organic substances on the surface and adsorptive properties of environmental black carbon (char): Attenuation of surface activity by humic and fulvic acids. *Environ. Sci. Technol.* **2006**, *40*, 7757–7763.
44. Kwon, S.; Pignatello, J.J. Effect of natural organic substances on the surface and adsorptive properties of environmental black carbon (char): Pseudopore blockage by model lipid components and its implication for N_2-probed surface properties of natural sorbents. *Environ. Sci. Technol.* **2005**, *39*, 7932–7939.
45. Lou, L.; Liu, F.; Yue, Q.; Chen, F.; Yang, Q.; Hu, B.; Chen, Y. Influence of humic acid on the sorption of pentachlorophenol by aged sediment amended with rice-straw biochar. *Appl. Geochem.* **2013**, *33*, 76–83.
46. Harms, H.; Bosma, T.N.P. Mass transfer limitation of microbial growth and pollutant degradation. *J. Ind. Microbiol. Biotechnol.* **1997**, *18*, 97–105.
47. Rhodes, A.H.; Carlin, A.; Semple, K.T. Impact of black carbon in the extraction and mineralisation of phenanthrene in soil. *Environ. Sci. Technol.* **2008**, *42*, 740–745.
48. Zhou, Z.; Sun, H.; Zhang, W. Desorption of polycyclic aromatic hydrocarbons from aged and unaged charcoals with and without modification of humic acids. *Environ. Pollut.* **2010**, *158*, 1916–1921.