The Impact of Enhanced and Non-Enhanced Biochars on the Catabolism of ^{14}C-Phenanthrene in Soil

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Abstract

Biochar is a by-product from the pyrolysis of biomass and has a great potential in soil amendment due to its carbon and nutrient-rich properties. The aim of this study was to investigate the impact of increasing amounts (0, 0.01, 0.1, 0.2, 0.5 and 1.0%) of two types of biochar (so-called enhanced and non-enhanced) to soil on the biodegradation of ^{14}C-phenanthrene. Enhanced biochar contains inoculants which are designed to potentially stimulate microbial activity and promote biological function in soil. After 100 d of incubation, the addition of 0.5% and 1% enhanced (EbioC) and non-enhanced biochars (NEbioC) led to longer lag phases, reduced rates and extents of ^{14}C-phenanthrene in amended soil. However, in soils amended with 0.01%, 0.1% and 0.2% amendments, extents of mineralisation of ^{14}C-phenanthrene increased and were found to be higher in the EBioC- as compared to the NEbioC-amended soils. Increasing soil-phenanthrene contact time also increased ^{14}C-phenanthrene mineralisation in soil which had received smaller amounts of EBioC. Application of both EbioC and NEbioC also enriched the soil microbial populations during the incubation. However, it was found that phenanthrene-degrading microbial populations declined as soil contact time increased; this was particularly true for soils receiving larger amounts of due to reduction in the mobile/bioaccessible fraction of the phenanthrene in soil. The findings revealed the importance of the type and amount of biochar that may be added to soil to stimulate or enhance organic contaminant biodegradation.

Keywords — Enhanced biochar; non-enhanced biochar; phenanthrene; mineralization; soil-PAH contact time

1. Introduction

The degree and impact of polycyclic aromatic hydrocarbons (PAHs) in soil pollution resulting from oil spills, especially from production and exploration activities (Obida et al., 2018), and other anthropogenic sources (Simon and Sobieraj, 2006); pose great threats to human health and the environment. These organic contaminants are known to be carcinogenic and mutagenic in nature (Dong et al., 2012). Microbial degradation is one of the most important mechanisms for removing these contaminants from soil, but the rate and extent of removal is dependent on the chemical and physical properties of the contaminant (Semple et al,. 2007; Simon and Sobieraj, 2006; Xie et al., 2015) as well the soil properties which include nutrient availability, temperature, moisture, presence and activity of the target degrading microorgansims, bioavailable fraction of the contaminant to the degrading microbes, and heterogeneity of the soils (organic matter and mineral fractions) and their associated pore structures (Okere and Semple, 2012; Riding et al., 2013; Semple et al., 2013; Umeh et al., 2017). The heterogenous nature of organic matter and mineral fractions in soil, in part, determines the sorption-desorption mechanisms of contaminants within the soil matrix (Umeh et al., 2017). These properties have contributed to a large extent to the hydrophobicity, lipophilicity, solubility, soil-water partition coefficient (Kd), persistence, mass transfer, mobility, bioaccessibility and biodegradation of the organic contaminants in soil (Abdel-Shafy and Mansour, 2016; Ghosal et al., 2016). In the case of PAHs, increases in the number of fused benzene rings will also increase the persistence as higher molecular weight PAHs are less biodegradable in soil (Couling et al., 2010; Abdel-Shafy and Mansour, 2016).

The application of microbial degradation for the clean up contaminated soils is called bioremediation, which depends on the intrinsic role of microbes and their metabolic enzymes to metabolize chemicals down to less toxic metabolites or to CO_2. However, using biodegradation as a tool to remediate contaminated soils is often too slow to reduce associated risk to acceptable levels; therefore, interventions are often required to speed up the process (Xu et al., 2018). Recent reports show that several studies have been carried out to enhance the remediation of contaminated soil but present several challenges, including low nutrients and contaminant bioavailability, a reduction in soil microbial activity and a low degradative potential by the indigenous microbes involved in the biodegradation process (Bisht et al., 2015; Zhang et al., 201 6; Kong et al., 2018). It is therefore imperative that the use of nutritional and biological enrichments to contaminated soils should be done through economically viable and environmentally sustainable approaches.

One approach to enhancing bioremediation involves the addition of biomass-derived materials to contaminated soil. Organic materials, such as biochars, may offer a low cost, carbon- and nutrient-rich biomass amendment for stimulating and/or enhancing biodegradation of PAHs in contaminated soil. Biochars are pyrolytic products from organic feedstocks under zero or low-oxygen concentrations at different temperatures of 250^{o}C –1000^{o}C (Kuśmierz et al., 2016; Kumar et al., 2018). Studies have shown that these carbonaceous and sorbent materials improved soil physicochemical properties (structure, stability, pH, water holding capacity, nutrients, carbon energy (Anyika et al., 2015); adsorb and retain nitrogen form (NH_4^+) in soil (Gai et al., 2014), while stimulating microbial activity, growth and composition

(Galitskaya et al., 2016), as well as changes in the microbial community structure in soil (Zhang et al., 2018). Additionally, biochar not only influences the oxygen level in soil but also supports aerobic and anaerobic biodegradation (Anyika et al., 2015), especially when used at low dose in biochar-treated soil. Apart from improving microbial activity, the sorptive properties of biochar gives an additional benefit of trapping contaminant in soil.

The sorptive properties of biochars have been widely studied, however few studies have also reported their biodegradative potential for PAHs in soils, owing to their stimulation for substrate bioavailability for microbial degradation through the formation of microhabitat (bacteria and fungi) for actively growing autochthonous soil microflora through electrostatic attraction and attachment to biochar's porous surfaces (Anyika et al., 2015; Ogbonnaya et al., 2016; Zhang et al., 2018). For example, a recent study showed that microbe-biochar interaction enhanced mass transfer of PAHs (making the contaminant more bioavailable) to immobilized cells, thus resulting to higher PAH degradation when compared to un-inoculated biochar in a sorbent-amended system (Xiong et al., 2017). Although the claims of the effectiveness of using biochar to improve bioremediation, its impact on soil bioremediation, depends on the concentration of the contaminant, soil conditions, active sites, types and properties of the biochar (Jones et al., 2012; Yuan et al., 2019). Biochars have been widely studied as a soil conditioner and immobilized carriers particularly for the biodegradation and management of soil remediation. However, the effects of biochar types (enhanced biochar and non-enhanced biochar) and application rate to soil for optimum PAHs metabolism have not been studied extensively.

120

121 The main objectives of this research study were (i) to investigate the impact of
122 biochars (enhanced and non-enhanced) on the catabolic potential in soil on ^{14}C-
123 phenanthrene mineralisation (a model PAH compound) over time; (ii) to determine
124 the effects of increasing amounts of both biochars on the mineralisation of ^{14}C-
125 phenanthrene in soil over time, and (iii) to estimate changes in microbial numbers in
126 biochar-amended and phenanthrene-spiked soil.

127

128

129 **2. Materials and Methods**

130 *2.1 Soil and Biochar*

131 The surface agricultural soil (5–20 cm depth) used for this study was collected
132 from Myerscough Agricultural College, Preston, United Kingdom. After transferring to
133 the laboratory, soil samples were air-dried, homogenized, sieved through a 2mm
134 mesh and thereafter, stored in the dark until use. Information on the soil
135 characteristics are presented in Table S1 (Couling et al., 2010). Processed biochars,
136 microbially enhanced (EbioC) and non-enhanced (NEbioC), were collected from a
137 biochar processing plant, Lancaster, UK. Biochars were produced from combined
138 lignocellulosic feedstocks (virgin wood and agricultural residues) by slow pyrolysis at
139 a high temperature of 500^{o}C for 4 h under a low oxygen atmosphere in a muffle
140 furnace. Some information on the physical and chemical properties of the biochars
141 are presented in Table 1. After production, some pyrolyzed biochars were cultured
142 (enriched) with mixed microbial inoculants: the arbuscular mycorrhizal fungi *such as*
143 *Glomus* spp (> 450 propagules/g), *Ascophyllum nodosum* and *Trichoderma* spp
144 (>1x10^{9} CFU/g), as well as wormcasts. Biological amendments were prepared and

immobilized (physically attached) onto the biochar surface by spraying onto the material, thereby producing the enhanced biochar. Both biochars were specifically produced for agricultural purposes as soil conditioners to provide required nutrients and to improve soil biological function for plant growth.

2.1. Biochar doses and soil amendment conditions

EbioC and NEbioC were amended to soil at different amounts: 0.0%, 0.01%, 0.1%, 0.2%, 0.5% and 1% (dry weight basis) per total mass of soil. The above doses were amended into the soil to evaluate the potential of each rate to stimulate microbial activities and optimal phenanthrene biodegradation. Soil moisture content (25% on dry matter basis) and pH (7.4 – 7.5) after soil-biochar amendments were monitored and maintained throughout the study period.

2.2. Soil spiking and Incubation conditions

Sieved and homogenized soil was spiked with ^{12}C-phenanthrene (100 mg/kg) to give a final concentration of 240 mg/kg; and soil spiking was done according to 'Bolus methodology' (Doick et al., 2003). Briefly, the whole soil sample was divided into four portions; one proportion (approx. 525 g) was spiked with ^{12}C-phenanthrene using acetone as the carrier solvent. This was closely followed by blending the remaining three equal parts of the soil, and afterward, allow to vent for 4hrs (in a fume cupboard) to evaporate acetone (Lee et al., 2003). This was done after rehydration with sterile water based on the water holding capacity (WHC) of soil (55 wt%), while maintaining 25% MC, however. The soil was then amended with different amounts of EbioC and NEbioC: 0.0%, 0.01%, 0.1%, 0.2%, 0.5% and 1% (w/w). All biochar-amended soil samples were prevented from photo-oxidation by

storage in air-tight separate amber glass bottles and further incubated in the dark at 21 ± 1°C for 0, 25, 50, 75 and 100 days. Triplicate mixtures were placed in an air-tight amber glass bottle for each condition and allowed to weather in the dark at 21 ± 2°C for 1, 25, 50, 75 and 100 days. Control (non-amended) soils were also incubated alongside with amended soils.

2.3. Mineralisation assay in biochar-amended soils

Respirometry assay was carried out to determine the catabolic evolution (^{14}C-phenanthrene to $^{14}CO_2$) from both biochars-amended soil according to standard methods (Reid et al., 2001; Semple et al., 2006). Briefly, the mineralisation of ^{14}C-phenanthrene was monitored in soil microcosms by weighing 10 ± 0.1 g (dry weight) from soil-biochar mixtures into 30 ml of sterile distilled water in 250 ml Scott bottles (Teflon-lined screw cap). Thereafter, the slurry was spiked with ^{14}C-radiolabelled phenanthrene standard (0.5 kBq per respirometer bottle) and incubated at 100 rpm on a flat-bed orbital shaker at 21 ± 1°C. Additionally, each bottle contained a CO_2 trap (7 ml vial) with 1 M NaOH and monitored bihourly for 1 d and then regularly for 24 hrs during an 18 d incubation period. Sampling was done at every time point (1, 25, 50, 75 and 100 d) for biochar-amended soils. Catabolic response in biochar-treated soils were assessed at each sampling period through the lag phases (when mineralisation reached 5%), the fastest rates of $^{14}CO_2$ evolved (expressed also as the maximum rate of $^{14}CO_2$ production) within 1 d in amended soils, and the total extents of ^{14}C-phenanthrene mineralisation after 18 d (Macleod and Semple, 2006).

2.4. Microbial enumeration analyses

Spread plate method was used to enumerate the total heterotrophs and phenanthrene degraders in biochar-amended soil (Okere and Semple, 2012; (Anyanwu and Semple, 2016). A 10-fold serial dilution was done by weighing 1 ± 0.01 g of biochar-amended soil in 9 ml of Ringer's solution (¼ strength). An aliquot was further transferred into appropriate plates. The plate count agar (PCA) supplemented with an antimicrobial agent (streptomycin-penicillin-glutamic, 8 µl/ml) and, amphotericin-B (5 µl/ml) added to potato dextrose agar (PDA) were used for enumeration of heterotrophic bacteria and fungi, respectively. Phenanthrene degraders (bacteria and fungi) were counted in minimal basal salt (MBS) media (Vázquez-Cuevas et al., 2018) incorporated with 0.05 mg/ml of ^{12}C-phenanthrene (as sole carbon source) and supplemented with appropriate antimicrobials (bacteria and fungi). Microbial numbers were quantified and presented in CFUs/g_{dw} soil.

2.5. Statistical analysis

The biochar-amended experiments in this study were carried out in triplicates. The data were analysed on Statistical Package for the Social Sciences (IBM SPSS Version 23.0). All amended soils were analysed using one-way analysis of variance (ANOVA) at 95% confidence level ($p < 0.05$) to determine the least significant difference (LSD). To test for the effects of both biochars and amounts in phenanthrene-spiked soils, comparison of means within and across time points were analysed using Tukey's Post-Hoc and Games-Howell test to ascertain differences in biochar-treated soils. Pearson product-moment correlation coefficient (r) was performed to describe the relationship between microbial numbers with the rate and extent of ^{14}C-phenanthrene mineralised in biochar-treated soils. The value of r is

ranked on a scale between +1 and –1 as clearly described elsewhere (Omoni et al., 2020).

3. Results

3.1. Mineralisation of ^{14}C-phenanthrene in biochar-amended soils

The impacts of both biochars (EbioC and NEbioC) on the mineralisation of ^{14}C-phenanthrene in soil were studied with varied amounts of biochar (0.0%, 0.01%, 0.1%, 0.2%, 0.5% and 1%) during 18 d soil respirometry after 1, 25, 50, 75 and 100 d of soil-PAH contact time (Fig. 1 and 2; Table 2 and 3). The lag phase represents the time taken to reach 5% mineralisation. The lag phases behaviour in the two treatment soils, EbioC and NEbioC were not obviously different from the non-treated soils in most time points throughout the study. Soil amended with EbioC produced shorter lag periods when compared to their counterpart soil (NEbioC). The shortest lag phase of 0.46 ± 0.00 d and 0.63 ± 0.00 d were achieved in the 0.1% EbioC and NEbioC-amended soils, respectively. On the other hand, the longest lag phase of 8.37 ± 0.01 d and 8.86 ± 0.03 d were achieved in the 0.01% EbioC and NEbioC-amended soils, respectively. However, after 1 d soil-PAH contact time, the biochar types showed significantly longer lag phases in all amended soils. The larger application doses (0.5 and 1.0%) displayed shorter lag phases compared to other treatments and control (0%). This trend was not consistent following further soil ageing, for example, the application doses (0.5 and 1.0%) for both EbioC and NEbioC greatly extended the length of the lag phase (50 > 75 > 100 d) ($p < 0.01$). However, in comparison to 1 d soil-PAH contact time, biochar application doses (0.01, 0.1, 0.2, 0.1 & 1.0%) after 25 d ageing period, displayed reductions in lag

phases with these orders of magnitude starting from the lowest to highest amount of EbioC (16 > 15 > 14 > 13 > 7-fold) and NEbioC (12 > 11 > 10 > 9 > 8-fold) (Table 2 and 3). Generally, EbioC (0.1 & 0.2%) consistently reduced the lag phases ($p < 0.001$) compared to other biochar dosages and unamended soil (control) throughout the study period; while soil amended with the NEbioC did not display any similar trend in amended soils (Table 2). Noticeably, the lag phases in the non-amended (control) soil were significantly shorter ($p < 0.05$) than those soils receiving 0.5 and 1.0% amendments for the NEbioC.

As observed for the lag phases, the addition of EbioC resulted in faster rates of ^{14}C-phenanthrene mineralisation than those observed in the NEbioC incubations (Tables 2 and 3). In addition, the fastest rates of mineralisation in both EbioC and NEbioC-amended soils were generally higher in the soils that had received the smaller amounts of biochar (0.1, 0.2 & 0.01%) and in more aged soils (1 d to 100 d). However, in 1 d aged soils containing 1% EbioC biochar, there was a significantly increase in the fastest rates of mineralisation compared to other soil amendments and control (Table 2). However, there were no significant changes ($p < 0.05$) in the 1% EbioC biochar from this point onward throughout the incubation. There were significantly faster rates of mineralisation ($p < 0.001$) observed in 0.1% EbioC- (2.29%/d) and NEbioC- (1.24%/d) amended soils compared to the other soil conditions (Table 2 and 3). Further, in soils with no biochar amendment, significantly faster rates of mineralisation were found at most time points compared to the NEbioC-amended soils.

As soil contact time increased in the biochar-amended soils, there were commensurate increases in the fastest rates of ^{14}C-phenanthrene mineralisation, especially in soils receiving smaller amounts (0.01, 0.1 & 0.2%) of biochar. Interestingly, the results for EbioC-amended soils showed that the lower amounts of biochar (0.01, 0.1 & 0.2%) resulted in increases the fastest rates of $^{14}CO_2$ mineralised by 80.7%, 83.4% and 79.4%, after 25 d, respectively, compared to 1-day soil-PAH contact time ($p < 0.001$). Similarly, the results also showed significant increases in fastest rates for 0.01, 0.1 and 0.2% biochar after 75-d (64.2, 21.1 and 38.8%) and 100 d (76.1, 68.1 and 82.2%) incubations when compared to 1-d soil incubation. Correspondingly, the fastest rates of mineralisation were found at 75 d for all NEbioC-amended soils; showing higher rates of 75.6% and 58.1% increases for 0.01 and 0.1% amendments, respectively, compared to 1-d incubation. However, in this investigation, when compared to both biochar-treated soils, the non-amended (0%) soil consistently showed increases in the fastest rates of mineralisation ($p < 0.001$) as soil-phenanthrene contact time increased: 51.8%, 67.1%, 82.1% and 71.3% after 1, 25, 50 and 100 d, respectively.

The effects of the catabolic potential of both EbioC and NEbioC and their respective dosage application to phenanthrene-spiked soil on the total extent of ^{14}C-phenanthrene mineralisation were also monitored (Figs. 1 and 2; Tables 2 and 3). Results indicate that soils amended with 0.1% and 0.2% amounts of biochar showed the highest extent of ^{14}C-phenanthrene mineralisation (45.6% and 32.57%), while the lowest were recorded for 1.0% (24.71% and 23.01%) with EbioC and NEbioC ($p < 0.001$), respectively. At the first time point (1 d), EbioC (0.1%) generally showed considerably higher extents of mineralisation within and across treatments and

293 control ($p < 0.001$) and over the rest of the incubation period. A similar trend was
294 observed for soils with NEbioC amendments, but these were not significantly
295 different from 0.01% biochar amendment ($p > 0.05$). Furthermore, after 75 and 100 d
296 soil-PAH contact time, the total extents of mineralisation significantly increased in the
297 0.1% (29.3 and 20.5%) and 0.2% (27.9 and 32.26%) EbioC-amended to soils
298 respectively, when compared to 1 d aging period. Greater amounts of biochar
299 amendment (0.5 and 1.0%) did not significantly increase ($p < 0.05$) the total extents
300 of ^{14}C-phenanthrene mineralisation throughout the study for either the EbioC or the
301 NEbioC soil amendments. In general, the presence of the NEbioC-biochar in PAH-
302 spiked soil did not influence the total extents of mineralisation positively compared to
303 EbioC; the exception being in the soils receiving smaller biochar amendments (0.01
304 and 0.1%).
305
306 *3.2. Microbial enumeration in biochar-amended soil*
307 *3.2.1 Bacterial numbers*
308 Soil amendment with biochar (EbioC and NEbioC) significantly increased the
309 total heterotrophic bacterial number at all time points ($p < 0.05$ *and* 0.001), as
310 compared to control (Tables 2 and 3). Further, EbioC-amended soils consistently
311 displayed similar patterns that could be represented in the order of increasing
312 heterotrophic bacterial number (50 > 75 > 100 > 25 > 1) ($p < 0.001$) compared to
313 NEbioC-amended soil. The heterotrophic bacterial numbers were also significantly
314 influenced by 0.1% biochar addition than all other soil amendment conditions (EbioC
315 and NEbioC), including the unamended soil ($p < 0.05$).
316

317 Similarly, the addition of both biochars to phenanthrene-spiked soil statistically
318 increased the number of phenanthrene degrading bacteria (CFUs g^{-1} soil) in all soil
319 conditions compared to control (unamended) throughout the study (Tables 2 and 3).
320 Increasing the soil contact time with PAH also significantly influenced ($p < 0.05$) the
321 CFUs in EbioC and NEbioC-amended soils; although these numbers were not
322 consistent in NEbioC-amended soil. The number of phenanthrene-degrading
323 bacteria for both biochar types in amended soils with lower amounts (0.01, 0.1 and
324 0.2%) presented much higher CFUs as compared to the higher biochar amended
325 soils (0.5 and 1.0%) and control soil ($p > 0.05$). Comparatively, after 1 day, EbioC
326 (0.1 and 0.2%) behaved differently in soil with 32 and 26-fold and 32, 16 and 19-fold
327 increase in phenanthrene-degrading bacterial numbers at 50-day, respectively, when
328 compared to other amendment conditions and incubation period. Similarly, the
329 numbers of phenanthrene degraders in the NEbioC-amended soils resulted in 32, 16
330 and 19-fold increase in amended soil (0.01, 0.1 and 0.2%), respectively.
331 Furthermore, as mentioned earlier, most contact points did not significantly stimulate
332 increases in the numbers of phenanthrene degraders ($p > 0.05$) when amended with
333 higher amounts of biochar (0.5 and 1.0%) in both soil amendments (EbioC and
334 NEbioC).
335
336 From the data, the number of phenanthrene degraders showed a moderately
337 positive linear correlation ($r = 0.55, p < 0.05$) and moderately negative correlation ($r =$
338 $-0.63, p < 0.05$) with total extent of ^{14}C-phenanthrene mineralisation for 0.2% and
339 1.0% of EbioC-amended soils, respectively. Also, a negative correlation for 0.1% ($r =$
340 $-0.54, p < 0.05$) and 0.2% ($r = -0.64, p < 0.05$) between phenanthrene-degraders and
341 total extent of mineralisation for NEbioC were observed, for the respective

342 application doses (Fig. S1). Phenanthrene-degraders displayed a significantly strong
343 but negative correlation ($r = -0.72, p < 0.01$) with fastest rates of ^{14}C-phenanthrene
344 mineralised in 1.0% NEbioC-amended soil; whilst the number of ^{14}C-phenanthrene
345 degraders observed in 0.1% and 0.5% showed a significant, but negative
346 correlations ($r = -0.52, p < 0.05$ and $r = -0.66, p < 0.05$) with fastest rates of
347 mineralisation, respectively (Fig. S3).
348
349 *3.2.2 Fungal numbers*
350 Overall, the numbers of phenanthrene-degrading fungi were inconsistent within
351 and across biochar-amended soils over time (Tables 2 and 3). The total fungal
352 numbers (TFCs) were significantly higher in the control soils compared to soils
353 amended with biochar at 50 d of exposure. However, this trend changed as both
354 biochar types added to soils significantly resulted in higher TFC after a longer
355 contact (75 to 100 days), resulting in a significant decreasing order of magnitude
356 (1.0% < 0.5% < 0.2% < 0.1% < 0.01%). Phenanthrene-degrading fungi recorded the
357 highest CFUs (7.97×10^4 g^{-1} soil dw) in 0.2% NEbioC-amendment after 25 d of
358 incubation**.** However, significant numbers of phenanthrene degrading fungi were also
359 recorded in soils amended with biochar (0.01% and 0.1%) compared to other
360 amendment conditions. A significant positive correlation was recorded between the
361 phenanthrene degraders with both fastest rates ($r = 0.80, p < 0.000$) and total
362 extents ($r = 0.52, p < 0.05$) of ^{14}C-phenanthrene mineralised in 0.2% EbioC-
363 amended soil, while 0.1% negatively correlated with both fastest rates ($r = 0.82, p <$
364 0.000) and total extents ($r = 0.60, p < 0.05$) of mineralisation in amended soils (Fig.
365 S2). Finally, NEbioC-amended soils with 0.01% and 1.0% amounts of biochar
366 showed strong positive correlations ($r = 0.72, p < 0.01$ and $r = 0.83, p < 0.01$)

between ^{14}C-phenanthrene-degraders and total extents of ^{14}C-phenanthrene mineralisation, respectively (Fig. S4).

4. Discussion

4.1. Effects of enhanced and non-enhanced biochar on ^{14}C-phenanthrene mineralisation

Addition of EbioC to PAH contaminated soil increased microbial activity and greatly impacted on ^{14}C-phenanthrene mineralisation when compared to NEbioC-amended soils, which was even more evident following increases in soil-phenanthrene contact time. This may be due to more bioaccessible fractions of the sorbed-PAH substrate on the immobilised biochar surface for biodegradation (Uyttebroek et al., 2006; Zhang et al., 2018), thus resulting in an increase in soil metabolic activity. Further, Xiong et al. (2017) showed that biochar incorporated into soil provided a protective niche allowing for higher metabolic activities and increases in the concentration gradients between sorbed-PAH on biochar surfaces and contact with microbial cell surfaces over shorter distances. Similarly, Galitskaya et al. (2016) also observed higher PAH fluxes and stimulated biodegradation in amended soil with biochar. Mass transfer of PAHs to degrading cells in a biochar-amended soil could be facilitated by dissolved and solid sorbing matrices for PAH degradation (Xiong et al., 2017).

The addition of external nutrient supplies and organic materials to soil would influence the indices of quantifying biodegradation, such as lag phases, fastest rates

392 and overall extents of organic contaminant biodegradation (Jablonowski et al., 2013;
393 Oyelami et al., 2013; Ogbonnaya et al., 2016). In this current study, the immobilised
394 inoculum on the EbioC could have provided an additional support for the indigenous
395 microbial population than NEbioC which led to shorter lag phases, increases in
396 fastest rates and extents of ^{14}C-phenanthrene mineralisation in amended soil. The
397 biological carrier materials (EbioC) can accelerate nutrient uptake and release,
398 improved oxygen and water holding capacity and support metabolic activities and
399 processes in soil rhizosphere (Yakhin et al., 2017; Drobek et al., 2019), thereby
400 indicating their efficacies in nutrient stimulatory action in a poor contaminated or
401 polluted agricultural soil.
402
403 Analysis of our results showed shorter lag phases, faster rates and greater
404 extents of mineralisation in the lower biochar doses (0.1% > 0.2% > 0.01%) of both
405 biochar type in amended soils; however, this was more pronounced in EbioC -
406 amended soils. Although, both biochar-amended soils showed significantly extended
407 lag phases after 1 d soil incubation, increasing soil-PAH contact time (25 d onward),
408 the lower biochar amendments (0.1% > 0.2% > 0.01%) impacted on the lag phases,
409 rates and extents of phenanthrene biodegradation in soil. This can be attributed to
410 the fraction of the phenanthrene that is rapidly desorbable or present in the aqueous
411 biochar amended soil phase, which is accessible to microbial cells and their
412 catabolic apparatus (Ogbonnaya et al., 2014; Rhodes et al., 2010).
413
414 The fastest rates of ^{14}C-phenanthrene mineralisation depended on the amounts
415 of biochar-amended to soil. Smaller amounts of biochar increased the rates of
416 mineralisation in comparison to the larger amendments over time in both biochar

417 types. Similarly, Ogbonnaya et al. (2014) also reported reductions in rates of
418 phenanthrene catabolism in soils which had received large amounts (1%) of wood-
419 derived biochar. It has been reported that properties such as porosity, contaminant
420 concentration and physico-chemical properties, organic matter content, cation
421 exchange capacity, soil contact time, soil properties, microbial activity, diversity and
422 dynamics may all influence rates of mineralisation of organic contaminants in soil
423 (Semple et al., 2003; Ogbonnaya et al., 2013). It is worthy of note that faster rates of
424 mineralisation were measured in soils receiving the EbioC-amendment compared to
425 the NEbioC-amended soils. This clearly indicates the potential of the inoculants in
426 stimulating microbial activity and contaminant catabolism in soil, in particular.

427

428 The application of larger amounts (1.0% > 0.5%) of both biochar types reduced
429 ^{14}C-mineralisation in soil. Even though biochar has been reported to have intrinsic
430 ability to biodegrade organic contaminants (Anyika et al., 2015), the sorptive
431 properties of black carbon, including biochars, can reduce mass transfer and
432 bioaccessibility of PAHs to soil microorganisms (Rhodes et al., 2008; 2012; Anyika et
433 al., 2015; Ogbonnaya et al., 2016). This further suggests the likely effect that is seen
434 in this investigation occurs in soils with higher biochar amendments. Biochar type
435 (quantity and quality), dose, and application conditions have also been reported
436 previously as some determining factors which govern the degree of sequestration of
437 PAHs in biochar-amended soil (Sopeña, et al., 2012; Galitskaya et al., 2016; Xiong
438 et al., 2017). For example, in this current study, biochar was prepared at a higher
439 temperature (~500 ^{o}C), which could have increase aromaticity, owing to associated
440 higher surface area and lower cation-exchange capacity (Anyika et al., 2015),

441 suggesting a greater sorptive effect of PAHs to surfaces as observed for larger
442 biochar amendments reported here (0.5 and 1.0%).

443

444 *4.2. Influence of microbial numbers on the mineralisation of ^{14}C-phenanthrene in*
445 *biochar-amended soil*

446

447 Soil amended with biochar can influence the abundance, diversity, and
448 distribution of soil microbial communities, owing to changes in their biological,
449 physical and chemical properties which might also lead to an increase/decrease in
450 soil microbial biomass, the minerals content and organics in soil (Awad et al., 2018).
451 The data obtained in this study revealed stimulated microbial numbers (both bacteria
452 and fungi) in EbioC-amended soils over time. Particularly, it was also found that the
453 lower amendments (0.01, 0.1 & 0.2%) of EbioC added to soil increase the bacterial
454 and fungal CFUs (heterotrophs and phenanthrene-degraders); whereas the 0.5%
455 and 1.0% biochar amendments resulted in lower CFUs especially from 50 d to 100 d
456 in NEbioC-amended soils. The numbers measured in the lower amendments are
457 likely be due to increasing metabolic rates, as seen in the fastest rates of ^{14}C-
458 phenanthrene mineralisation, bioavailable nutrients, lower microbial community
459 disruption and lower water vapour isotherms as a result of reduced soil organic
460 matter (Peake et al., 2014). Soil minerals and organic matter can sorb and bind to
461 biochar surfaces, thus creating more and potentially stronger sorption sites in the
462 biochar for organic contaminants (Semple et al., 2007; Ogbonnaya and Semple,
463 2013). It may be hypothesised that the phenanthrene may have been more strongly
464 sorbed to the soil-biochar complex in the soils which received larger amendments of
465 0.5 and 1.0%. This may have caused a slower or irreversible desorption of the PAH

or rapidly depleted desorbable fractions of the contaminant over time (50 -100 d) for microbial uptake and biodegradation (Rhodes et al., 2008; 2012). For example, Rhodes et al. (2012) noticed a 7.8-fold decrease in rapidly ($\%F_{rap}$) and a corresponding increase in slowly ($\%F_{slow}$) desorbing fractions of phenanthrene in soils receiving larger amount of black carbon (a form of pyrolyzed carbon) and with increase soil contact time (1 d to 100 d). Further, Rhodes et al. (2012) found that there were 50% reductions in the rates of ^{14}C-phenanthrene mineralised with increasing amount of black carbon from 0 to 5% after 20 d soil-PAH contact time. Therefore, it can be suggested that the greater amounts of biochar increased the number of microporous sites for contaminant sorption in biochar-amended soils. Generally, the biochar increased the total numbers of heterotrophs (bacteria and fungi) but caused a decrease in phenanthrene degraders, suggesting reduced bioaccessibility/desorbable (biodegradable) fractions of the PAH studied, which is important for the growth, proliferation and contaminant uptake and metabolism by the PAH-degraders in soil.

It has been concluded that the biodegradation of PAHs in contaminated soil occurs through the actions of microbial consortia rather than by a single microbial population, although this depends on their catabolic potential and enzymes for substrates sequestration (Oyelami et al., 2013; Gupta et al., 2016). In this present study, significant negative correlations were observed between phenanthrene degraders and mineralisation (rates and extents) for most biochar amendments to the soil. This may be as a result of either the microbes are not synthesizing the required enzymes for the target contaminant (however, this was not measured) or they were partially or fortuitously metabolizing the phenanthrene in the presence of

non-target carbon substrate (in this case, the biochar) (Namkoong et al., 2002; Das et al., 2011). However, phenanthrene-degrading microorganisms quantified in 0.2% of EbioC and NEbioC positively correlated with rates and extents of mineralisation, respectively; while 0.01% and 1.0% doses also showed a positive correlation between phenanthrene-degrading fungi and rates of mineralisation for NEbioC-amended soils only. Such correlations could explain the effects and behaviour of different amounts of biochar in the amended soils (Khorram et al., 2016). Therefore, the presence of PAH degrading populations may not be too adequate to indicate the degradation of a contaminant in soil but their high CFUs could contribute to the extent of PAH degradation and provide also useful information on the degradability of the contaminant by indigenous microorganisms in soils.

4. Conclusions

The overall findings in this study show that EbioC amendments stimulated the mineralisation of ^{14}C-phenanthrene in soils to greater levels than the NEbioC amendments. Biochar application and soil-contaminant contact time influenced the lag phase, rates and extents of ^{14}C-phenanthrene mineralised, which were particularly evident in the lower amendments (0.1%, 0.2%, 0.01%) in both types of biochar. Enhanced and non-enhanced wood-derived biochars influenced the microbial numbers and catabolic activity in phenanthrene contaminated soil. Phenanthrene-degrading microbial populations markedly reduce with increased biochar-soil contact time and in soils receiving higher amounts of biochar. The findings reported here show that smaller amounts of biochar caused increases in fastest rates and extents of ^{14}C-phenanthrene mineralisation, especially with the enhanced biochar. The reduced rates and extents of ^{14}C-phenanthrene

516 mineralisation found in the soil receiving larger amounts (0.5 and 1.0%) of biochar
517 are likely to be attributed to increase in the number of sites for contaminant sorption
518 in soil-biochar complexes, thus reducing the bioavailability/bioaccessibility of the
519 target contaminant. Therefore, larger amounts of biochar may well inhibit the
520 removal of organic contaminants from the soil. However, both situations have the
521 potential to reduce the risk associated with PAHs, either through loss by stimulating
522 biodegradation or by reducing the PAH mobility and/or bioaccessibility in soil.
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524
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530 **References**
531 Abdel-Shafy, H.I., Mansour, M.S.M., 2016. A review on polycyclic aromatic
532 hydrocarbons: Source, environmental impact, effect on human health and
533 remediation. Egypt. J. Pet. 25, 107–123.
534 https://doi.org/10.1016/j.ejpe.2015.03.011
535 Anyanwu, I.N., Semple, K.T., 2016. Effects of phenanthrene and its nitrogen-
536 heterocyclic analogues aged in soil on the earthworm *Eisenia fetida*. Appl. Soil
537 Ecol. 105, 151–159. https://doi.org/10.1016/j.apsoil.2016.04.014
538 Anyika, C., Abdul Majid, Z., Ibrahim, Z., Zakaria, M.P., Yahya, A., 2015. The impact
539 of biochars on sorption and biodegradation of polycyclic aromatic hydrocarbons
540 in soils—a review. Environ. Sci. Pollut. Res. 22, 3314–3341.

https://doi.org/10.1007/s11356-014-3719-5

Awad, Y.M., Ok, Y.S., Abrigata, J., Beiyuan, J., Beckers, F., Tsang, D.C.W., Rinklebe, J., 2018. Pine sawdust biomass and biochars at different pyrolysis temperatures change soil redox processes. Sci. Total Environ. 625, 147–154. https://doi.org/10.1016/j.scitotenv.2017.12.194

Bisht, S., Pandey, P., Bhargava, B., Sharma, S., Kumar, V., Krishan, D., 2015. Bioremediation of polyaromatic hydrocarbons (PAHs) using rhizosphere technology. Braz. J. Microbiol. 46, 7–21. https://doi.org/10.1590/S1517-838246120131354

Couling, N.R., Towell, M.G., Semple, K.T. 2010. Biodegradation of PAHs in soil: Influence of chemical structure, concentration and multiple amendment. Environ. Pollut. 158, 3411–3420. https://doi.org/10.1016/j.envpol.2010.07.034

Das, C., Bhowal, A., Datta, S., 2011. Bioremediation of Copper-Contaminated Soil by Co-Application of Bioaugmentation and Biostimulation with Organic Nutrient. Biorem. J. 15, 90–98. https://doi.org/10.1080/10889868.2011.570283

Doick, K.J., Lee, P.H., Semple, K.T. 2003. Assessment of spiking procedures for the introduction of a phenanthrene-LNAPL mixture into field-wet soil. Environ. Pollut. 126, 399–406. https://doi.org/10.1016/S0269-7491(03)00230-6

Dong, C. Di, Chen, C.F., Chen, C.W., 2012. Determination of polycyclic aromatic hydrocarbons in industrial harbor sediments by GC-MS. Int. J. Environ. Res. Public Health 9(6), 2175–2188. https://doi.org/10.3390/ijerph9062175

Drobek, M., Frąc, M., Cybulska, J., 2019. Plant Biostimulants: Importance of the Quality and Yield of Horticultural Crops and the Improvement of Plant Tolerance to Abiotic Stress—A Review. Agron. 9, 335. https://doi.org/10.3390/agronomy9060335

566 Gai, X., Wang, H., Liu, J., Zhai, L., Liu, S., Ren, T., Liu, H., 2014. Effects of
567 feedstock and pyrolysis temperature on biochar adsorption of ammonium and
568 nitrate. PLoS ONE, 9, 1–19. https://doi.org/10.1371/journal.pone.0113888
569 Galitskaya, P., Akhmetzyanova, L., Selivanovskaya, S., 2016. Biochar-carrying
570 hydrocarbon decomposers promote degradation during the early stage of
571 bioremediation. Biogeosci. 13, 5739–5752. https://doi.org/10.5194/bg-13-5739-
572 2016
573 Ghosal, D., Ghosh, S., Dutta, T.K., Ahn, Y., 2016. Current state of knowledge in
574 microbial degradation of polycyclic aromatic hydrocarbons (PAHs): A review.
575 Front. Microbiol. 7:1369. https://doi.org/10.3389/fmicb.2016.01369
576 Gupta, K.K., Aneja, K.R., Rana, D., 2016. Current status of cow dung as a
577 bioresource for sustainable development. Bioresour. Bioprocess. 3, 28.
578 https://doi.org/10.1186/s40643-016-0105-9
579 Jablonowski, N.D., Borchard, N., Zajkoska, P., Fernández-Bayo, J.D., Martinazzo,
580 R., Berns, A.E., Burauel, P., 2013. Biochar-mediated [^{14}C] atrazine
581 mineralization in atrazine-adapted soils from Belgium and Brazil. J. Agri. Food.
582 Chem. 61, 512–516. https://doi.org/10.1021/jf303957a
583 Jones, D.L., Rousk, J., Edwards-Jones, G., DeLuca, T.H., Murphy, D.V., 2012.
584 Biochar-mediated changes in soil quality and plant growth in a three year field
585 trial. Soil Biol. Biochem. 45, 113–124.
586 https://doi.org/10.1016/j.soilbio.2011.10.012
587 Kong, L., Gao, Y., Zhou, Q., Zhao, X., Sun, Z., 2018. Biochar accelerates PAHs
588 biodegradation in petroleum-polluted soil by biostimulation strategy. J. Hazard.
589 Mater. 343, 276–284. https://doi.org/10.1016/j.jhazmat.2017.09.040
590 Kumar, S., Pattnaik, D., Bhuyan, S. K., Mishra, S.C., 2018. Influence of Temperature

591 on Pyrolysis of Biomass. IOP Conference Series: Mater. Sci. Eng. 338, 012061
592 https://doi.org/10.1088/1757-899X/338/1/012061
593 Kuśmierz, M., Oleszczuk, P., Kraska, P., Pałys, E., Andruszczak, S., 2016.
594 Persistence of polycyclic aromatic hydrocarbons (PAHs) in biochar-amended
595 soil. Chemosphere 146, 272–279.
596 https://doi.org/10.1016/j.chemosphere.2015.12.010
597 Lee, P.H., Doick, K.J., Semple, K.T., 2003. The development of phenanthrene
598 catabolism in soil amended with transformer oil. FEMS Microbiol. Lett. 228,
599 217–223. https://doi.org/10.1016/S0378-1097(03)00751-1
600 Macleod, C.J.A., Semple, K.T., 2006). The influence of single and multiple
601 applications of pyrene on the evolution of pyrene catabolism in soil. Environ.
602 Pollut. 139, 455–460. https://doi.org/10.1016/j.envpol.2005.06.014
603 Namkoong, W., Hwang, E.Y., Park, J.S., Choi, J.Y., 2002. Bioremediation of diesel-
604 contaminated soil with composting. Environment. Pollut. 119, 23–31.
605 https://doi.org/10.1016/S0269-7491(01)00328-1
606 Obida, C.B., Alan Blackburn, G., Duncan Whyatt, J., Semple, K.T., 2018. Quantifying
607 the exposure of humans and the environment to oil pollution in the Niger Delta
608 using advanced geostatistical techniques. Environ. Int. 111, 32–42.
609 https://doi.org/10.1016/j.envint.2017.11.009
610 Ogbonnaya, U.O., Thomas, J., Fasina, S.A., Semple, K.T., 2016. Impact of two
611 contrasting biochars on the bioaccessibility of ^{14}C-naphthalene in soil. Environ.
612 Technol. Innovation 6, 80–93. https://doi.org/10.1016/j.eti.2016.07.002
613 Ogbonnaya, U., Oyelami, A., Matthews, J., Adebisi, O., Semple, K., 2014. Influence
614 of wood biochar on phenanthrene catabolism in soils. Environ. 1, 60–74.
615 https://doi.org/10.3390/environments1010060

616 Ogbonnaya, U., Semple, K., 2013. Impact of Biochar on Organic Contaminants in
617 Soil: A Tool for Mitigating Risk? Agron. 3, 349–375.
618 https://doi.org/10.3390/agronomy3020349

619 Okere, U.V, Semple, K.T., 2012. Biodegradation of PAHs in 'Pristine' Soils from
620 Different Climatic Regions. J. Biorem. Biodegrad. S1:006.
621 https://doi.org/10.4172/2155-6199.S1-006

622 Okere, U.V., Schuster, J.K., Ogbonnaya, U.O., Jones, K.C., Semple, K.T., 2017.
623 Indigenous ^{14}C-phenanthrene biodegradation in "pristine" woodland and
624 grassland soils from Norway and the United Kingdom. Environ. Sci. Processes
625 Impacts 19, 1437–1444. https://doi.org/10.1039/c7em00242d

626 Omoni, V.T., Lag-Brotons, A.J., Semple, K.T., 2020. Impact of organic amendments
627 on the development of 14C-phenanthrene catabolism in soil. Int. Biodeter.
628 Biodegrad. 151, 104991. https://doi.org/10.1016/j.ibiod.2020.104991

629 Oyelami, A.O., Okere, U V., Orwin, K.H., De Deyn, G.B., Jones, K.C., Semple, K.T.,
630 2013. Effects of plant species identity, diversity and soil fertility on
631 biodegradation of phenanthrene in soil. Environ. Pollut. 173, 231–237.
632 https://doi.org/10.1016/j.envpol.2012.09.020

633 Peake, L.R., Reid, B.J., Tang, X., 2014. Quantifying the influence of biochar on the
634 physical and hydrological properties of dissimilar soils. Geoderma 235–236,
635 182–190. https://doi.org/10.1016/j.geoderma.2014.07.002

636 Reid, B.J., Macleod, C.J.A., Lee, P.H., Morriss, A.W.J., Stokes, J.D., Semple, K.T.
637 (2001). A simple ^{14}C-respirometric method for assessing microbial catabolic
638 potential and contaminant bioavailability. FEMS Microbiol. Lett. 196, 141–146.

639 Rhodes, A.H., Carlin, A., Semple, K.T., 2008. Impact of black carbon in the
640 extraction and mineralization of phenanthrene in soil. Environ. Sci. Technol. 42,
641 740–745. https://doi.org/10.1021/es071451n

642 Rhodes, A.H., McAllister, L.E., Semple, K.T., 2010. Linking desorption kinetics to
643 phenanthrene biodegradation in soil. Environ. Pollut. 158, 1348–1353.
644 https://doi.org/10.1016/j.envpol.2010.01.008

645 Rhodes, A.H., Owen, S.M., Semple, K.T., 2007. Biodegradation of 2,4-
646 dichlorophenol in the presence of volatile organic compounds in soils under
647 different vegetation types. FEMS Microbiol. Lett. 269, 323–330.
648 https://doi.org/10.1111/j.1574-6968.2007.00657.x

649 Rhodes, A.H., Riding, M.J., Mcallister, L.E., Lee, K., Semple, K.T., 2012. Influence of
650 activated charcoal on desorption kinetics and biodegradation of phenanthrene in
651 soil. Environ. Sci. Technol. 46, 12445−12451.
652 https://doi.org/10.1021/es3025098

653 Riding, M.J., Doick, K.J., Martin, F.L., Jones, K.C., Semple, K.T., 2013. Chemical
654 measures of bioavailability/bioaccessibility of PAHs in soil: Fundamentals to
655 application. J. Hazard. Mater. 261, 687–700.
656 https://doi.org/10.1016/j.jhazmat.2013.03.033

657 Safaei Khorram, M., Zhang, Q., Lin, D., Zheng, Y., Fang, H., Yu, Y., 2016. Biochar: A
658 review of its impact on pesticide behavior in soil environments and its potential
659 applications. J. Environ. Sci. 44, 269–279.
660 https://doi.org/10.1016/j.jes.2015.12.027

661 Semple, K.T., Dew, N.M., Doick, K.J., Rhodes, A.H., 2006. Can microbial
662 mineralization be used to estimate microbial availability of organic contaminants

663 in soil ? Environ. Pollut. 140, 164–172.
664 https://doi.org/10.1016/j.envpol.2005.06.009

665 Semple, K.T., Doick, K.J., Wick, L.Y., Harms, H., 2007. Microbial interactions with
666 organic contaminants in soil: Definitions, processes and measurement. Environ.
667 Pollut. 150, 166–176. https://doi.org/10.1016/j.envpol.2007.07.023

668 Semple, K.T., Morriss, A.W.J., Paton, G.I., 2003. Bioavailability of hydrophobic
669 organic contaminants in soils: fundamental concepts and techniques for
670 analysis. Eur. J. Soil Sci. 54, 809–818. https://doi.org/10.1046/j.1365-
671 2389.2003.00564.x

672 Semple, K.T., Riding, M.J., Mcallister, L.E., Sopena-vazquez, F., Bending, G.D.
673 (2013). Impact of black carbon on the bioaccessibility of organic contaminants in
674 soil. J. Hazard. Mater. 261, 808–816.
675 https://doi.org/10.1016/j.jhazmat.2013.03.032

676 Simon, J. A., Sobieraj, J.A., 2006. Contributions of common sources of polycyclic
677 aromatic hydrocarbons to soil contamination. Rem. 16, 25–35.
678 https://doi.org/10.1002/rem.20089

679 Sopeña, F., Semple, K., Sohi, S., Bending, G., 2012. Assessing the chemical and
680 biological accessibility of the herbicide isoproturon in soil amended with biochar.
681 Chemosphere 88, 77–83. https://doi.org/10.1016/j.chemosphere.2012.02.066

682 Umeh, A.C., Duan, L., Naidu, R., Semple, K.T., 2017. Residual hydrophobic organic
683 contaminants in soil: Are they a barrier to risk-based approaches for managing
684 contaminated land? Environ. Int. 98, 18–34.
685 https://doi.org/10.1016/j.envint.2016.09.025

686 Uyttebroek, M., Breugelmans, P., Janssen, M., Wattiau, P., Joffe, B., Karlson, U.,
687 Ortega-Calvo, J.J., Bastiaens, L., Ryngaert, A., Hausner, M., Springael, D.,
688 2006. Distribution of the Mycobacterium community and polycyclic aromatic
689 hydrocarbons (PAHs) among different size fractions of a long-term PAH-
690 contaminated soil. Environ. Microbiol. 8, 836–847.
691 https://doi.org/10.1111/j.1462-2920.2005.00970.x
692 Vázquez-Cuevas, G.M., Stevens, C.J., Semple, K.T., 2018. Enhancement of ^{14}C-
693 phenanthrene mineralisation in the presence of plant-root biomass in PAH-
694 NAPL amended soil. Int. Biodeter. Biodegrad. 126, 78–85.
695 https://doi.org/10.1016/j.ibiod.2017.09.021
696 Xie, T., Reddy, K.R., Wang, C., Yargicoglu, E., Spokas, K., 2015. Characteristics
697 and applications of biochar for environmental remediation: A review. Crit. Rev.
698 Environ. Sci. Technol. 45, 939–969.
699 https://doi.org/10.1080/10643389.2014.924180
700 Xiong, B., Zhang, Y., Hou, Y., Arp, H.P.H., Reid, B.J., Cai, C., 2017. Enhanced
701 biodegradation of PAHs in historically contaminated soil by M. gilvum inoculated
702 biochar. Chemosphere 182, 316–324.
703 https://doi.org/10.1016/j.chemosphere.2017.05.020
704 Xu, X., Liu, W., Tian, S., Wang, W., Qi, Q., Jiang, P., Gao, X., Li, F., Li, H., Yu, H.,
705 2018. Petroleum hydrocarbon-degrading bacteria for the remediation of oil
706 pollution under aerobic vonditions: A perspective analysis. Front. Microbiol. 9,
707 1–11. https://doi.org/10.3389/fmicb.2018.02885
708 Yakhin, O.I., Lubyanov, A.A., Yakhin, I.A., Brown, P.H., 2017. Biostimulants in Plant
709 Science: A global perspective. Front. Plant Sci. 7, 2049.
710 https://doi.org/10.3389/fpls.2016.02049

711 Yuan, P., Wang, J., Pan, Y., Shen, B., Wu, C., 2019. Review of biochar for the
712 management of contaminated soil: Preparation, application and prospect.
713 Sci.Total Environ. 659, 473–490. https://doi.org/10.1016/j.scitotenv.2018.12.400
714 Zhang, G., Guo, X., Zhu, Y., Liu, X., Han, Z., Sun, K., Ji, L., He, Q., Han, L., 2018.
715 The effects of different biochars on microbial quantity, microbial community shift,
716 enzyme activity, and biodegradation of polycyclic aromatic hydrocarbons in soil.
717 Geoderma 328, 100–108. https://doi.org/10.1016/j.geoderma.2018.05.009
718 Zhang, H., Tang, J., Wang, L., Liu, J., Gurav, R.G., Sun, K., 2016. A novel
719 bioremediation strategy for petroleum hydrocarbon pollutants using salt tolerant
720 *Corynebacterium variabile* HRJ4 and biochar. J. Environ. Sci. 47, 7–13.
721 https://doi.org/10.1016/j.jes.2015.12.023
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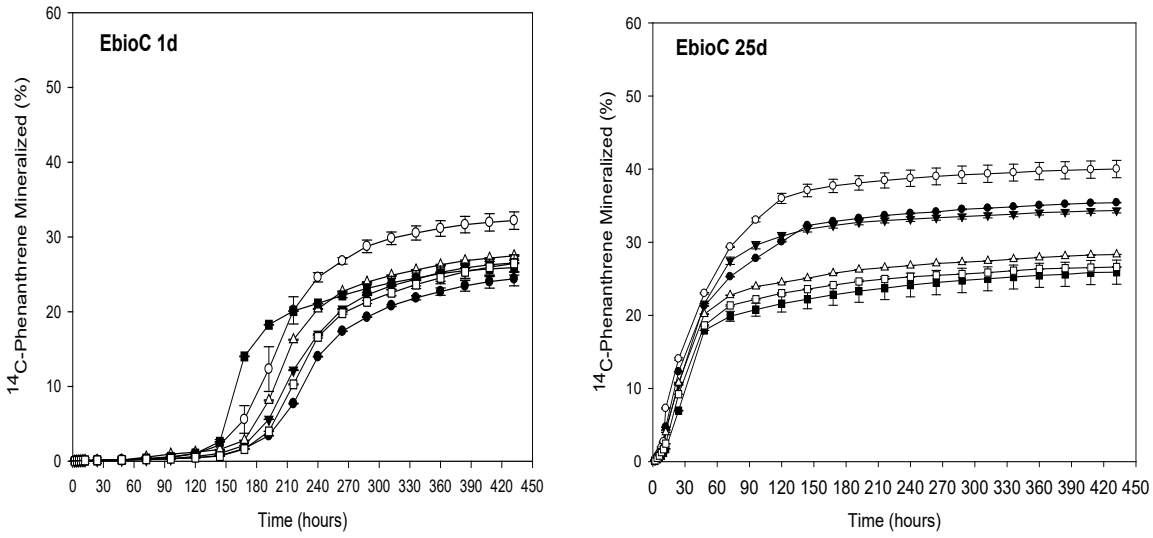
724 **Figure Legends**

725 **Figure 1.** Evolution of $^{14}CO_2$ from the catabolism of ^{14}C-phenanthrene in soil
726 (100mg/kg) amended with enhanced biochar at 0.01% (●), 0.10% (○), 0.20% (▼),
727 0.50% (∆), 1% (■) and Control (□) after 1, 25, 50d soil-phenanthrene contact time.
728 Standard error of mineralisation (SEM) are represented as triplicate samples (n = 3).

729

730 **Figure 2.** Evolution of $^{14}CO_2$ from the catabolism of ^{14}C-phenanthrene in soil
731 (100mg/kg) amended with non-enhanced biochar at 0.01% (●), 0.10% (○), 0.20%
732 (▼), 0.50% (∆), 1% (■) and Control (□) after 1, 25, 50d soil-phenanthrene contact
733 time. Standard error of mineralisation (SEM) are represented as triplicate samples (n
734 = 3).

735 **Figure 1**



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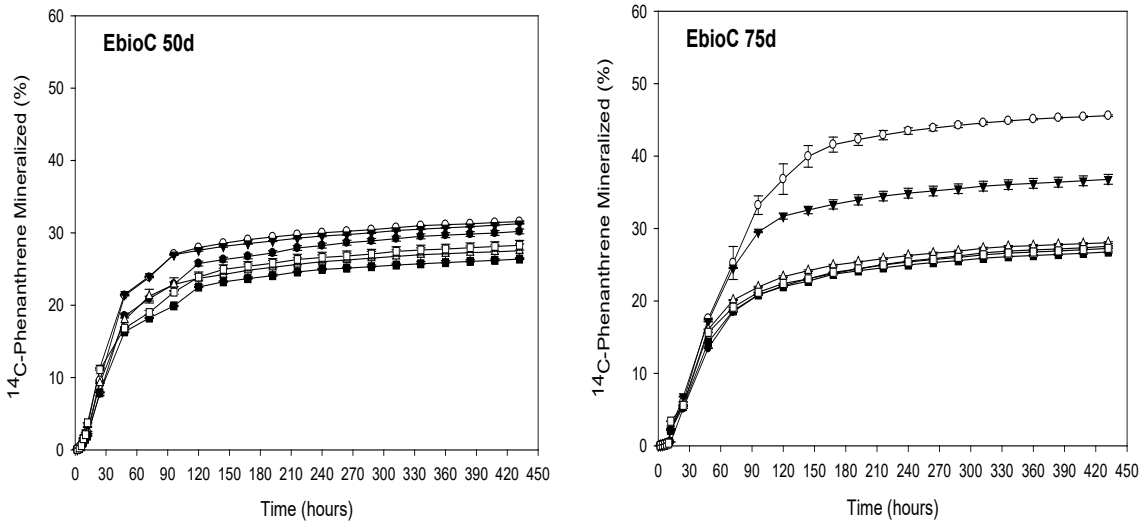

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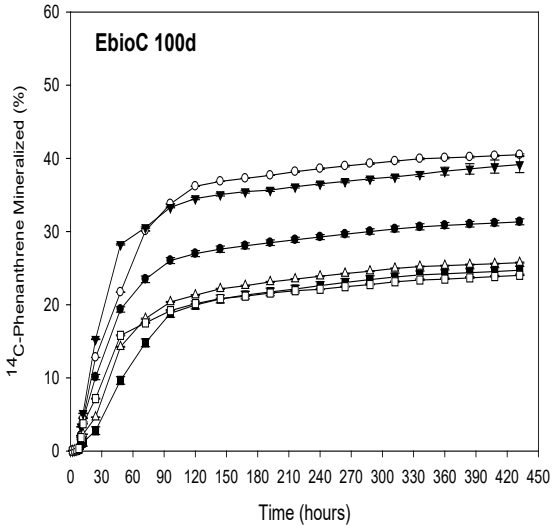
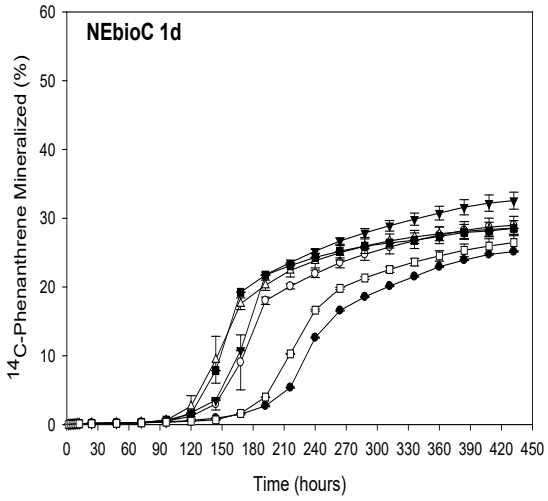
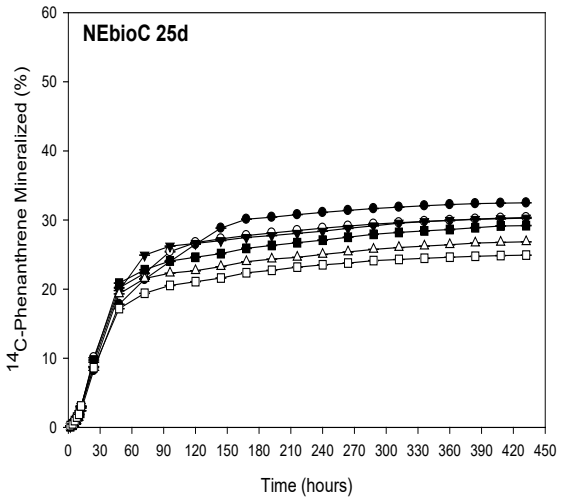


738 **Figure 2**





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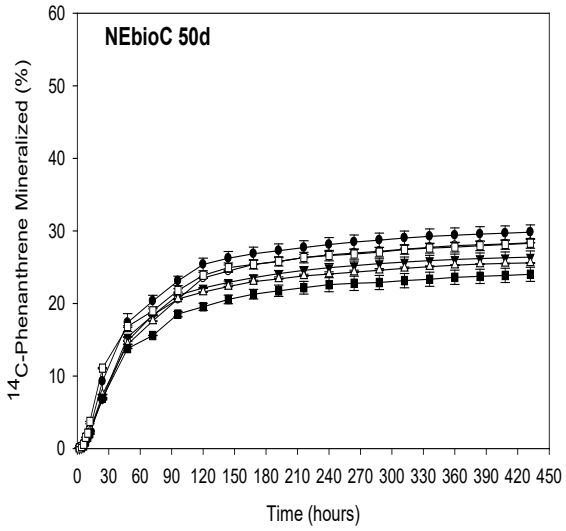

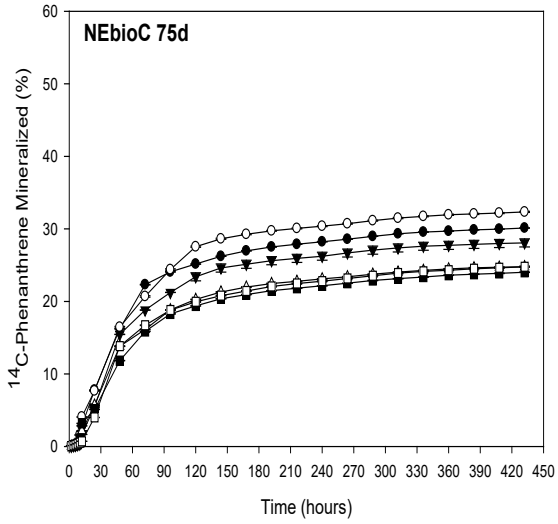

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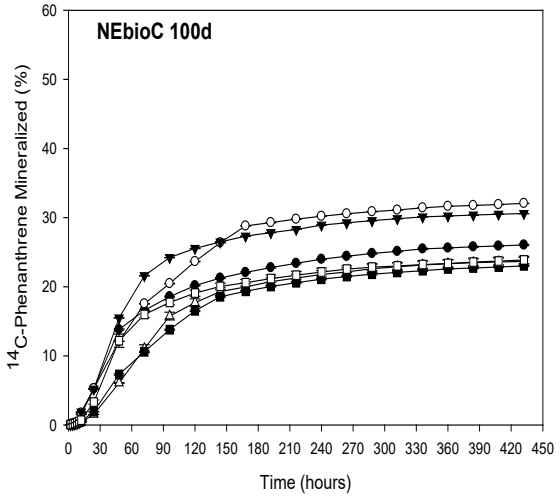

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743 **Table Legends**

744

745 **Table 1.** Physical and chemical properties of biochars used in the experiment

746

747 **Table 2.** Catabolic and autochthonous microbial number profile (CFU/g) of ^{14}C-
748 phenanthrene mineralisation in soil amended with varying amounts of **enhanced**
749 biochar. Values are mean ± standard error (n = 3).

750

751 **Table 3.** Catabolic and autochthonous microbial number profile (CFU/g) of ^{14}C-
752 phenanthrene mineralisation in soil amended with varying amounts of **non-**
753 **enhanced** biochar. Values are mean ± standard error (n = 3).

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769 Table 1. Physical and chemical properties of the biochars used in the experiment

Parameters analyzed		Enhanced Biochar	Non-enhanced biochar	Instruments used and methodologies described
pH		8.6	9.0	pH meter (Jenway model 3504 Bench combined) (**Li et al., 2013**)
EC (µS/cm)		1136	498	Conductivity meter (Jenway model 3504 Bench combined), (**Li et al., 2013**)
TC (%)		50.4	78.2	Schimadzu TOC-L analyser (**Siudek et al., 2015**)
Total N (dry wt) (% dry)		1.15	1.55	Elemental analyser (Vario EL III CHNOS, Hanau, Germany), (**Wilke, 2010**)
NH_4-N (%)		0.12	0.032	Autoanalyzer model 3HR (AAR 3HR), (**Haney et al., 2008**).
Ash (%)		35.6	14	Fisher Isotemp 650 Model 58 Programmable Muffle Furnace (**Domingues et al., 2017).**
Total solids (%)		85.1	66.6	Thermophilic oven (Genlab, UK), (**Marmiroli et al.,2018**)
Total P (%)		0.10	0.045	Spectrophometer (**Limwikran et al., 2019)**
Mineral components (wt %)				Inductively coupled plasma spectrometry (ICS–MS, PerkinElmer NexION 2000 (**Limwikran et al., 2019**)
	Total Ca	5.85	5.17	
	Total Mg	0.23	0.17	
	Total K	0.72	0.56	
	Total Na	0.12	0.015	
	Total B	0.002	0.002	
	Total Fe	0.27	0.012	
	Total Mn	0.009	0.002	
	Total Al	0.19	0.015	
	Total S	0.14	0.041	
	Total Mo	-	-	
	Total Cl	0.084	0.019	
	Total Cu	-	-	
	Total Zn	0.002	-	

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771 * EC = electrical conductivity, TC = Total carbon, NH_4-N (ammonium-nitrogen), P = phosphorus, Ca =calcium, Mg = magnesium, K = potassium, Na =
772 sodium, B = boron, Fe = iron, Mn = manganese, Al = aluminium, S = sulphur, Mo = molybdenum, Cl = chlorine, Cu = copper, Zn = zinc

Table 2. Catabolic and autochthonous microbial number profile (CFU/g) of ^{14}C-phenanthrene mineralisation in soil amended with varying amounts of enhanced biochar. Values are mean ± standard error (n = 3).

					Bacteria		Fungi	
Soil-PAH aging (d)	Amended amounts	Lag phase (d)	Fastest rates (/d)	Total Extent (%)	Total Heterotrophs	PAH degraders	Total Heterotrophs	PAH degraders
					CFU x 10^7 g^{-1} soil dw		CFU x 10^4 g^{-1} soil dw	
1	0	8.16 ± 0.00	0.27 ± 0.00	26.47 ± 0.16	1.66 ± 0.03	0.93 ± 0.02	4.33 ± 0.12	2.13 ± 0.09
	0.01	8.37 ± 0.01	0.26 ± 0.00	24.40 ± 0.94	0.97 ± 0.02	0.83 ± 0.01	2.30 ± 0.12	3.07 ± 0.03
	0.1	7.22 ± 0.01	0.38 ± 0.00	32.20 ± 1.17	1.72 ± 0.03	0.94 ± 0.02	1.87 ± 0.09	3.23 ± 0.12
	0.2	7.82 ± 0.00	0.27 ± 0.00	26.52 ± 0.08	1.43 ± 0.02	0.87 ± 0.02	1.97 ± 0.09	2.23 ± 0.03
	0.5	7.41 ± 0.01	0.34 ± 0.00	27.50 ± 0.08	1.23 ± 0.03	0.59 ± 0.01	2.67 ± 0.12	2.27 ± 0.03
	1	6.21 ± 0.00	0.48 ± 0.00	25.91 ± 0.99	1.17 ± 0.02	1.09 ± 0.01	3.27 ± 0.15	2.93 ± 0.03
25	0	0.69 ± 0.00	0.56 ± 0.00	26.61 ± 0.03	7.17 ± 0.15	0.98 ± 0.01	4.77 ± 0.07	7.40 ± 0.10
	0.01	0.52 ± 0.00	1.35 ± 0.00	35.42 ± 0.05	12.4 ± 0.27	1.20 ± 0.01	2.40 ± 0.06	3.33 ± 0.12
	0.1	0.46 ± 0.00	2.29 ± 0.00	40.20 ± 1.18	19.0 ± 0.35	1.47 ± 0.02	2.40 ± 0.10	2.33 ± 0.07
	0.2	0.55 ± 0.00	1.31 ± 0.00	34.34 ± 0.50	14.3 ± 0.21	1.23 ± 0.01	2.37 ± 0.03	3.47 ± 0.09
	0.5	0.57 ± 0.00	1.15 ± 0.00	28.33 ± 0.02	14.9 ± 0.31	0.77 ± 0.01	5.17 ± 0.09	4.17 ± 0.09
	1	0.81 ± 0.00	0.46 ± 0.00	25.91 ± 1.65	14.6 ± 0.35	1.29 ± 0.02	4.07 ± 0.03	6.10 ± 0.06
50	0	0.59 ± 0.00	0.82 ± 0.00	28.29 ± 0.09	18.1 ± 0.15	2.95 ± 0.03	10.3 ± 0.22	6.12 ± 0.26
	0.01	0.75 ± 0.01	0.47 ± 0.01	30.20 ± 0.34	75.1 ± 0.42	28.2 ± 0.93	3.54 ± 0.19	4.09 ± 0.17
	0.1	0.67 ± 0.00	0.58 ± 0.00	31.56 ± 0.08	38.8 ± 0.42	31.0 ± 0.44	4.22 ± 0.26	3.80 ± 0.15
	0.2	0.61 ± 0.00	0.68 ± 0.02	31.26 ± 0.05	83.1 ± 1.12	23.4 ± 0.42	3.16 ± 0.15	3.04 ± 0.13
	0.5	0.70 ± 0.00	0.58 ± 0.00	27.53 ± 0.94	73.0 ± 0.84	3.44 ± 0.02	5.15 ± 0.30	4.39 ± 0.11
	1	0.76 ± 0.00	0.50 ± 0.00	26.38 ± 0.67	61.6 ± 1.12	3.64 ± 0.02	4.47 ± 0.33	3.63 ± 0.04
75	0	0.87 ± 0.00	1.51 ± 0.00	27.26 ± 0.06	15.4 ± 0.08	2.34 ± 0.02	3.38 ± 0.04	2.93 ± 0.07
	0.01	0.98 ± 0.00	0.39 ± 0.00	27.55 ± 0.04	56.1 ± 1.52	3.61 ± 0.01	5.06 ± 0.07	3.37 ± 0.07
	0.1	0.86 ± 0.00	0.94 ± 0.02	45.56 ± 0.16	88.2 ± 1.84	15.5 ± 0.22	6.14 ± 0.11	3.10 ± 0.12
	0.2	0.80 ± 0.00	0.93 ± 0.03	36.79 ± 0.67	53.2 ± 1.93	11.5 ± 0.23	5.27 ± 0.15	2.33 ± 0.07
	0.5	0.90 ± 0.01	0.90 ± 0.00	28.05 ± 0.05	42.2 ± 1.52	3.33 ± 0.02	4.18 ± 0.15	1.97 ± 0.09
	1	0.93 ± 0.01	0.92 ± 0.03	26.76 ± 0.03	43.9 ± 2.95	2.35 ± 0.01	4.09 ± 0.15	2.57 ± 0.12
100	0	0.69 ± 0.04	0.94 ± 0.00	24.02 ± 0.04	1.57 ± 0.02	2.94 ± 0.03	0.35 ± 0.00	2.91 ± 0.15
	0.01	0.53 ± 0.01	1.09 ± 0.04	31.30 ± 0.42	28.7 ± 0.19	20.8 ± 0.30	8.23 ± 0.15	3.21 ± 0.21
	0.1	0.52 ± 0.00	1.19 ± 0.04	40.50 ± 1.09	29.7 ± 0.37	22.8 ± 0.34	14.1 ± 0.13	2.74 ± 0.04
	0.2	0.49 ± 0.00	1.52 ± 0.02	39.15 ± 0.11	18.8 ± 0.15	29.4 ± 0.32	9.37 ± 0.26	3.54 ± 0.13
	0.5	1.08 ± 0.01	0.93 ± 0.00	25.75 ± 0.07	17.7 ± 0.11	3.16 ± 0.01	3.00 ± 0.21	2.83 ± 0.15
	1	1.27 ± 0.01	0.49 ± 0.00	24.71 ± 0.53	17.2 ± 0.38	18.9 ± 0.11	3.42 ± 0.26	1.94 ± 0.04

777 Table 3. Catabolic and autochthonous microbial number profile (CFU/g) of ^{14}C-phenanthrene mineralisation in soil amended with varying amounts of non-
778 enhanced biochar. Values are mean ± standard error (n = 3).

					Bacteria		**Fungi**	
Soil-PAH aging (d)	Amended amounts	Lag phase (d)	Fastest rates (/d)	Total Extent (%)	Total Heterotrophs	PAH degraders	Total Heterotrophs	PAH degraders
					CFU x 10^7 g^{-1} soil dw		CFU x 10^4 g^{-1} soil dw	
1	0	8.16 ± 0.00	0.27 ± 0.00	26.47 ± 0.13	0.66 ± 0.01	0.93 ± 0.01	4.33 ± 0.12	2.13 ± 0.09
	0.01	8.86 ± 0.04	0.30 ± 0.00	25.15 ± 0.09	1.10 ± 0.01	0.92 ± 0.01	2.30 ± 0.11	3.30 ± 0.11
	0.1	7.02 ± 0.06	0.52 ± 0.01	28.98 ± 1.31	1.86 ± 0.02	1.01 ± 0.00	1.93 ± 0.03	3.10 ± 0.06
	0.2	6.20 ± 0.01	0.46 ± 0.00	32.57 ± 1.24	1.43 ± 0.00	0.88 ± 0.03	2.10 ± 0.06	2.53 ± 0.03
	0.5	5.80 ± 0.00	0.44 ± 0.00	28.57 ± 1.10	1.29 ± 0.02	0.56 ± 0.00	2.67 ± 0.12	2.03 ± 0.03
	1	5.55 ± 0.01	0.47 ± 0.00	28.54 ± 0.02	2.06 ± 0.03	1.15 ± 0.02	3.37 ± 0.09	4.17 ± 0.09
25	0	0.69 ± 0.00	0.56 ± 0.00	26.61 ± 0.03	7.17 ± 0.09	0.98 ± 0.01	4.77 ± 0.07	7.40 ± 0.10
	0.01	0.72 ± 0.00	0.49 ± 0.00	32.49 ± 0.02	9.47 ± 0.09	1.19 ± 0.01	2.40 ± 0.06	5.23 ± 0.09
	0.1	0.65 ± 0.00	0.60 ± 0.01	30.36 ± 0.06	12.0 ± 0.03	1.33 ± 0.02	2.33 ± 0.03	7.00 ± 0.06
	0.2	0.65 ± 0.00	0.54 ± 0.00	30.27 ± 0.05	6.53 ± 0.09	1.07 ± 0.01	2.23 ± 0.07	7.94 ± 0.03
	0.5	0.68 ± 0.00	0.57 ± 0.00	26.84 ± 0.01	9.87 ± 0.07	1.06 ± 0.01	4.70 ± 0.06	5.47 ± 0.03
	1	0.65 ± 0.00	0.71 ± 0.02	29.19 ± 0.04	8.84 ± 0.07	1.28 ± 0.01	4.83 ± 0.09	7.30 ± 0.12
50	0	0.59 ± 0.00	0.82 ± 0.00	28.29 ± 0.67	18.1 ± 0.17	2.95 ± 0.03	10.3 ± 0.22	6.12 ± 0.26
	0.01	0.78 ± 0.00	0.46 ± 0.02	29.83 ± 1.00	37.4 ± 0.26	20.9 ± 0.28	5.06 ± 0.13	3.88 ± 0.18
	0.1	0.79 ± 0.00	0.39 ± 0.00	28.37 ± 0.04	33.3 ± 0.26	17.0 ± 0.50	4.26 ± 0.15	4.01 ± 0.11
	0.2	0.79 ± 0.00	0.39 ± 0.00	26.38 ± 0.85	45.0 ± 0.11	16.9 ± 0.38	7.22 ± 0.34	4.85 ± 0.11
	0.5	0.76 ± 0.00	0.42 ± 0.00	25.60 ± 0.04	41.2 ± 0.11	3.12 ± 0.11	3.97 ± 0.11	4.77 ± 0.04
	1	0.80 ± 0.00	0.40 ± 0.00	24.12 ± 0.98	25.9 ± 0.30	3.38 ± 0.11	3.97 ± 0.08	3.12 ± 0.18
75	0	0.87 ± 0.00	1.51 ± 0.03	27.26 ± 0.06	15.4 ± 0.08	2.34 ± 0.02	3.38 ± 0.04	2.93 ± 0.07
	0.01	0.72 ± 0.00	1.23 ± 0.03	30.11 ± 0.02	46.0 ± 2.57	0.34 ± 0.02	2.95 ± 0.04	3.00 ± 0.06
	0.1	0.63 ± 0.00	1.24 ± 0.00	32.35 ± 0.07	66.7 ± 1.84	1.11 ± 0.02	4.73 ± 0.11	3.43 ± 0.09
	0.2	0.94 ± 0.00	0.66 ± 0.00	27.51 ± 0.03	43.5 ± 0.42	0.37 ± 0.03	2.83 ± 0.04	2.83 ± 0.07
	0.5	0.90 ± 0.01	0.90 ± 0.00	24.79 ± 0.05	47.3 ± 1.84	0.22 ± 0.03	2.70 ± 0.11	2.67 ± 0.12
	1	0.97 ± 0.01	1.05 ± 0.03	24.02 ± 0.03	38.8 ± 0.42	0.22 ± 0.04	2.78 ± 0.15	2.27 ± 0.03
100	0	0.69 ± 0.04	0.94 ± 0.02	24.02 ± 0.04	1.57 ± 0.02	2.94 ± 0.03	0.35 ± 0.00	2.91 ± 0.15
	0.01	0.97 ± 0.01	0.59 ± 0.00	26.05 ± 0.10	12.4 ± 0.15	2.65 ± 0.35	9.32 ± 0.24	2.70 ± 0.17
	0.1	0.96 ± 0.00	0.69 ± 0.03	32.06 ± 0.03	10.4 ± 0.19	4.73 ± 0.32	11.9 ± 0.28	3.71 ± 0.11
	0.2	0.97 ± 0.00	0.79 ± 0.00	30.59 ± 0.01	10.7 ± 0.37	7.34 ± 0.15	17.5 ± 0.22	2.36 ± 0.08
	0.5	1.76 ± 0.01	0.22 ± 0.01	23.67 ± 0.57	14.6 ± 0.38	2.04 ± 0.06	16.6 ± 0.26	2.24 ± 0.15
	1	1.57 ± 0.01	0.22 ± 0.00	23.01 ± 0.10	13.7 ± 0.15	1.96 ± 0.06	28.4 ± 0.26	3.08 ± 0.08

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