

1 **ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY**

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3 **Impact of nitrogen-polycyclic aromatic hydrocarbons on phenanthrene and benzo[a]pyrene**  
4 **mineralisation in soil**

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24 **Abstract**

25 When aromatic hydrocarbons are present in contaminated soils, they often occur in mixtures. The impact of  
26 four different (3-ring) nitrogen-containing polycyclic aromatic hydrocarbons (N-PAHs) on  $^{12/14}\text{C}$ -phenanthrene  
27 and  $^{12/14}\text{C}$ -benzo[a]pyrene (B[a]P) mineralisation in soil was investigated over a 90 d incubation period. The  
28 results revealed that both  $^{12/14}\text{C}$ -phenanthrene and  $^{12/14}\text{C}$ -benzo[a]pyrene showed no significant mineralisation  
29 in soils amended with  $10\text{ mg kg}^{-1}$  and  $100\text{ mg kg}^{-1}$  N-PAHs ( $p>0.05$ ). However, increases in lag-phases and  
30 decreases in the rates and extents of mineralisation were observed, over time. Among the N-PAHs,  
31 benzo[h]quinoline impacted  $^{14}\text{C}$ -phenanthrene mineralisation with extended and diauxic lag phases.  
32 Furthermore,  $^{12/14}\text{C}$ -B[a]P and  $^{14}\text{C}$ -benzo[a]pyrene–nitrogen-containing polycyclic aromatic hydrocarbons ( $^{14}\text{C}$ -  
33 B[a]P-N-PAHs) amended soils showed extensive lag phases ( $>21\text{ d}$ ); with some  $^{14}\text{C}$ -B[a]P-N-PAH  
34 mineralisation recording  $<1\%$  in both concentrations ( $10\text{ mg kg}^{-1}$  and  $100\text{ mg kg}^{-1}$ ), over time. This study  
35 suggests that the presence of N-PAHs in contaminated soil may impact the microbial degradation of polycyclic  
36 aromatic hydrocarbons (PAHs) and the impact was most likely the result of limited success in achieving  
37 absolute biodegradation of some PAHs in soil.

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39 **Keywords:** Mineralisation, N-PAHs, benzo[a]pyrene, phenanthrene, bioavailability.

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## 49 1. Introduction

50 Exploitation of the biodegradative capabilities of microorganisms for the clean-up of contaminated land has  
51 become widely accepted as an alternative to both physical and chemical methods because of its comparatively  
52 low cost and environmental sustainability (Sayer and Ripp, 2000; Sutherland et al., 2009; Doley et al., 2017;  
53 Menga et al., 2017; Li et al., 2017). Polycyclic aromatic hydrocarbons (PAHs) are among the most abundant  
54 organic contaminants with a potential to be degraded. Thus, attention has been paid to the degradation of PAHs  
55 and their catabolic pathways are well documented. However, most of these investigations on PAHs  
56 mineralisation have focused on single organic contaminants (Grosser et al., 1991; Yoon et al., 2002; Chauhan  
57 et al., 2008; Rhodes et al., 2008; Menga et al., 2017; Li et al., 2017). Studies have shown that PAHs degradation  
58 processes result in the formation of carbon dioxide (CO<sub>2</sub>), water (H<sub>2</sub>O) and simple inorganic compounds  
59 (maleic acid, fumaric acid, maleanic acid, glutaric acid, acetoacetyl-coenzyme). However, the kinetic efficiency  
60 of the pathway, type of reaction and bio-transformed metabolites produced depends on the number of aromatic  
61 rings and only limited success has so far been achieved in the biodegradation of some PAHs (Kästner and  
62 Mahro, 1996; Juhasz and Naidu, 2000; Lundstedt et al., 2003; Bamforth and Singleton, 2005; Menga et al.,  
63 2017; Doley et al., 2017). The reasons for the limited success are not well understood but could be constrained  
64 by one or more of the following process: (a) the solubilization of the PAHs, (b) their transport into the cell, (c)  
65 the expression of the degradative genes (d) the enzymatic breakdown of the PAHs (e) bioavailability and (f)  
66 the presence of other contaminants (Semple et al., 2007; Chauhan et al., 2008; Anyanwu and Semple, 2015b;  
67 Menga et al., 2017). Furthermore, interactions between low molecular weight (LMW) and high molecular  
68 weight (HMW) PAHs and their metabolites have been reported to play an important role in the induction of the  
69 catabolic enzymes, and such interactions can be either synergistic or antagonistic (Demanèche et al., 2004;  
70 Mohan et al., 2006; Couling et al., 2010). In the former case (synergistic), the metabolites produced in the  
71 degradation of aromatics in one strain may enhance the induction of catabolic enzymes of other aromatics in  
72 other strain(s) (cross-induction) (Whitman et al., 1998; Couling et al., 2010; Doley et al., 2017). In the latter  
73 case (antagonistic), aromatics or their metabolites may affect degradation due to substrate competition and/or  
74 microbial toxicity (Demanèche et al., 2004; Granato et al., 2017). Yet, the impact of more polar and soluble  
75 aromatics (such as 3-ring N-PAHs) on LMW and HMW PAHs mineralisation is not well known.

76 In a similar way, studies on biodegradation of nitrogen-containing polycyclic aromatics in soil have focussed  
77 mainly on indole, quinoline, isoquinoline, carbazole and acridine (Pereira et al., 1988; Fetzner, 1998; Millette  
78 et al., 1995; Lundstedt et al., 2003; Salam et al., 2017). However, biodegradation of the phenanthrolines (1,10-

79 phenanthroline, 1,7-phenanthroline, 4,7-phenanthroline and benzo[h]quinoline) have not been recorded in  
80 literature.

81 Nitrogen-containing polycyclic aromatic hydrocarbons (N-PAHs) which are not as much reported can exist,  
82 often at high concentrations in soil (Webber, 1994; Švábenský et al., 2009). N-PAH contamination, emanating  
83 from petrogenic, pyrogenic and biogenic processes, constitutes major pollution and toxicological problems  
84 within the environment (National Toxicology Program, 2001; US Environmental Protection Agency, 2001;  
85 IPCS Environmental Health Criteria, 2003; Hazardous Substance Data Bank, 2010; Environment Canada,  
86 2011; IARC, 2012; US Environmental Protection Agency, 2017). Despite the physico-chemical properties of  
87 N-PAHs, there is lack of information on the biodegradative impact of N-PAHs on LMW and HMW PAHs in  
88 the soil environment, over time.

89 Biodegradation is a major route of loss for organic contaminants in soil. It is widely known that polluted sites  
90 contain mixtures of compounds which vary in their impact to soil microbial community and as well,  
91 biodegradation of other aromatics. Irrespective of this, the impact of 3-ring N-heterocyclic aromatics (1,10-  
92 phenanthroline, 1,7-phenanthroline, 4,7-phenanthroline and benzo[h]quinoline) on phenanthrene and  
93 benzo[a]pyrene mineralisation has not been systematically studied in the soil environment. Conceptually,  
94 biodegradation of PAHs in soil by indigenous microbial communities may be enhanced or inhibited by the  
95 presence of other chemicals in contaminated sites. Thus, this study investigated if the presence, concentration  
96 and contact time of a more polar and soluble heterocyclic aromatics (3-ring N-PAHs) could enhance  
97 phenanthrene and B[a]P mineralisation in soil.

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## 99 **2. Materials and Methods**

### 100 *2.1. Chemicals*

101 Phenanthrene (Phen), 1,10-phenanthroline (1,10-Phen), 1,7-phenanthroline (1,7-Phen), 4,7-  
102 phenanthroline (4,7-Phen), benzo[h]quinoline (B[h]Q), benzo[a]pyrene (B[a]P) (Table 1) were obtained from  
103 Sigma-Aldrich, UK and <sup>14</sup>C-phenanthrene, <sup>14</sup>C-benzo[a]pyrene was obtained from ARC, UK. Goldstar liquid  
104 scintillation cocktail was supplied by Meridian Biotechnologies Ltd, UK.

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### 106 *2.2. Soil preparation*

107 Soil from Myerscough Agricultural College, UK was prepared for this study. The soil was collected  
108 from a depth of approximately 5 – 20 cm. The soil texture was sandy-loam (19.5% clay, 60.4% sand, 20.0%  
109 silt); organic matter content 2.7% and pH 6.5 (Doick et al., 2003). The soil was air dried at room temperature,  
110 sieved through a 2mm mesh and rehydrated back to 45% of the water holding capacity. Soil was amended with  
111 PAHs and N-PAH compounds using the method reported in Doick et al. (2003). Soils were placed in bowls:  $\frac{1}{3}$   
112 of the soil (100 g, n = 3) was spiked with individual standards of phenanthrene, B[a]P, benzo[h]quinoline, 1,10-  
113 phenanthroline, 1,7-phenanthroline or 4,7-phenanthroline dissolved in toluene (2 ml) to give concentrations of  
114  $10 \text{ mg kg}^{-1}$  and  $100 \text{ mg kg}^{-1}$  dry soil. The carrier solvent was allowed to volatilise from the soil for 3 – 4 h in  
115 a fume hood, after which soils were mixed with the remaining  $\frac{2}{3}$  of the soil (200 g). Samples were prepared  
116 using soils amended with toluene (only) to serve as a control. The amended soils were then kept in amber glass  
117 jars (500 ml) and incubated in the dark at  $21 \pm 1^\circ\text{C}$  for 1, 30, 60 and 90 d. Soil moisture content was checked  
118 regularly and lost water was replenished with deionized water. Recoveries of phenanthrene and its nitrogen-  
119 containing analogues from soil over time has been reported (Anyanwu and Semple, 2016a, b; 2015b).

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### 121 2.3. Mineralisation of $^{14}\text{C}$ -PAHs in soil

122 The ability of soil indigenous microflora to mineralise  $^{14}\text{C}$ -PAHs to  $^{14}\text{CO}_2$  was assessed at 1, 30, 60  
123 and 90 d soil-contact time. Respirometric assays were carried out in modified 250 ml Schott bottles  
124 incorporating a Teflon-lined screw cap containing 1 M NaOH to trap any  $^{14}\text{CO}_2$ , 10 g (n = 3) of soil (wet wt)  
125 and 30 ml sterile minimal basal solution (MBS) (Doick and Semple, 2003). Standards prepared in toluene to  
126 give a  $^{12}\text{C}$ -PAH concentration of  $10 \text{ mg kg}^{-1}$  and  $100 \text{ mg kg}^{-1}$  dry weight with an associated  $^{14}\text{C}$ -activity of 85  
127 Bq  $\text{g}^{-1}$  soil dry weight was then added. Analytical blanks consisted of soil with no  $^{14}\text{C}$ -amendment. A slurry  
128 system with a solid: liquid ratio of 1:3 was used to ensure complete  $^{12/14}\text{C}$ -PAH / N-PAH distribution (Doick  
129 and Semple, 2003). Respirometers were shaken at 100 rpm on an orbital shaker (Janke and Kunkel, IKA®-  
130 Labortechnik KS 510D) in the dark at  $21 \pm 1^\circ\text{C}$  and sampled every 24 h for 21 d. Sampling comprised of  
131 removal of vials containing trapped  $^{14}\text{CO}_2$  and replacement, followed by addition of 5 ml goldstar scintillation  
132 fluid. The vials were stored in the dark for 24 h before sample quantification were carried out by liquid  
133 scintillation counting (LSC) using standard calibration and quench correction techniques (Reid et al., 2001).

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## 135 2.4. Statistical analysis

136 Following blank correction, data were statistically analysed using SigmaStat 3.5 version. Effect of  
137 exposure time (incubation time), concentration and impact of N-PAHs on the mineralisation  $^{12/14}\text{C}$ -  
138 phenanthrene and  $^{12/14}\text{C}$ -benzo[a]pyrene was assessed using analysis of variance (ANOVA) to ascertain the  
139 significant differences in lag phases, fastest rates and extents of mineralisation. Differences were found to be  
140 statistically significant when  $p < 0.05$ . Data was presented as mean  $\pm$  standard error (SE) and graphs were plotted  
141 with SigmaPlot 10.0 version.

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## 143 3. Results

### 144 3.1. Mineralisation $^{12/14}\text{C}$ -phenanthrene in soil

145 Following the addition of the  $^{14}\text{C}$ -phenanthrene at 1 d, the indigenous microbial communities were  
146 able to rapidly mineralise  $^{14}\text{C}$ -phenanthrene in Phenanthrene amended soil as indicated by the relatively short  
147 lag phases of 2.77 d and 3.14 d in the 10 mg kg<sup>-1</sup> and 100 mg kg<sup>-1</sup> amendments over time ( $p > 0.05$ ) (Figs 1 and  
148 2). The extent of mineralisation was measured (Tables 2 and 3), and there were significant decreases in the  
149 extent to which  $^{12/14}\text{C}$ -phenanthrene was mineralised in soil over time compared with the N-PAH amendments  
150 ( $p < 0.05$ ). For example, decreases in mineralisation was recorded with increased soil-chemical-contact time  
151 (Tables 2 and 3). Furthermore, the rates of mineralisation showed that the mineralisation of  $^{12/14}\text{C}$ -phenanthrene  
152 amended soils recorded highest rate of  $0.87 \pm 0.00\% \text{ h}^{-1}$  and  $0.75 \pm 0.30\% \text{ h}^{-1}$  in the 10 mg kg<sup>-1</sup> and 100 mg  
153 kg<sup>-1</sup> amendments (1 d), respectively (Tables 2 and 3).

154 On the other hand, mineralisation of  $^{14}\text{C}$ -phenanthrene in the presence of N-PAHs occurred  
155 after long lag phases, over time. From the result, soils amended with the nitrogen-containing compounds  
156 exhibited extended lag phases of 3.05 d – 5.33 d ( $p > 0.05$ ) in the mineralisation of  $^{14}\text{C}$ -phenanthrene compared  
157 with phenanthrene amended soil over time (Figs 1 and 2). Among the N-containing aromatic chemicals,  
158 benzo[h]quinoline (B[h]Q) recorded the longest lag phase of 5.33 d at 1 d in the 100 mg kg<sup>-1</sup> amendment (Fig.  
159 2). In addition, B[h]Q displayed a diauxic lag phase of 4.55 d after reaching 5% mineralisation in the 100 mg  
160 kg<sup>-1</sup> amendment at 60 d (Fig. 2). The extents of mineralisation were measured, and N-PAHs showed no  
161 consistent trend in the mineralisation of  $^{14}\text{C}$ -phenanthrene in soil, over time. The extent of mineralisation

162 recorded high and low values in N-PAH soils over the 90 d incubation. However, low mineralisation values  
163 were recorded in both concentrations at 1 d and 60 d; with the exception of B[h]Q amended soils (Tables 2 and  
164 3).

165 The rates of mineralisation were also measured among the N-PAHs (Tables 2 and 3). N-containing  
166 polycyclic aromatics recorded low rates in the mineralisation of  $^{14}\text{C}$ -phenanthrene in soil over time. The rates  
167 of mineralisation showed that all the compounds recorded their highest mineralisation value at 1 d. For example,  
168 B[h]Q recorded the highest rate of  $0.90 \pm 0.10\% \text{ h}^{-1}$  ( $10 \text{ mg kg}^{-1}$ ); while 4,7-Phen recorded the highest value  
169 of  $0.77 \pm 0.10\% \text{ h}^{-1}$  ( $100 \text{ mg kg}^{-1}$ ) at 1 d, after extended lag phases of 3.43 d and 3.05 d, respectively (Tables  
170 1 and 2; Figs 1 and 2). 1,10-Phen recorded the lowest rates of  $0.17 \pm 0.10\% \text{ h}^{-1}$  in the  $10 \text{ mg kg}^{-1}$  amendment  
171 ( $60 \text{ d}$ ) and  $0.25 \pm 0.10\% \text{ h}^{-1}$  in the  $100 \text{ mg kg}^{-1}$  amendment ( $30 \text{ d}$ ), after long lag phases of 4.45 d and 4.91 d,  
172 respectively (Tables 1 and 2; Figs 1 and 2). From the study, a trend of increased lag phases, diauxic lag phases,  
173 slow rates of mineralisation and reduced extents of mineralisation of  $^{14}\text{C}$ -phenanthrene were observed in soils  
174 amended with N-PAHs in both concentrations, at all the time points. Statistical analysis of data showed  
175 significant difference in the lag phases over time ( $p < 0.05$ ). However, ANOVA revealed that N-PAHs had no  
176 significant positive impact on the mineralisation of  $^{14}\text{C}$ -phenanthrene in soil (over the 1 – 90 d soil-contact  
177 time) ( $p > 0.05$ ), with the exception of 4,7-Phen ( $100 \text{ mg kg}^{-1}$ ) ( $p < 0.05$ ).

178

### 179 *3.2. Mineralisation of $^{12/14}\text{C}$ -benzo[a]pyrene in soil*

180 Benzo[a]pyrene (B[a]P) amended soils followed similar pattern of increased lag phases ( $>21 \text{ d}$ ) in all  
181 the amendments and concentrations, at all the time points. The measured rates and extents of mineralisation  
182 showed that  $^{12/14}\text{C}$ -B[a]P rates of mineralisation recorded no mineralization in the  $10 \text{ mg kg}^{-1}$  and  $100 \text{ mg kg}^{-1}$   
183 ( $^{12/14}\text{C}$ -B[a]P amended soil at 30 d (Table 2). In the  $^{14}\text{C}$ -B[a]P–N-PAHs amendments, few N-PAHs recorded  
184 mineralisation rates of  $0.01 \pm 0.00\% \text{ h}^{-1}$  at varying days in the  $10 \text{ mg kg}^{-1}$  and  $100 \text{ mg kg}^{-1}$ , however, this was  
185 not statistically significant ( $p > 0.05$ ) (Tables 2 and 3). The extents of mineralisation were also measured and the  
186 result showed that most  $^{14}\text{C}$ -B[a]P–N-PAHs recorded values  $<0.1\%$  in the microbial mineralisation of  $^{14}\text{C}$ -  
187 B[a]P in both concentrations, over time. However, 4,7-Phen ( $10 \text{ mg kg}^{-1}$ ) and 1,10-Phen ( $100 \text{ mg kg}^{-1}$ )  
188 recorded mineralisation values  $>0.80\%$  at 1 d and 30 d, respectively (Tables 2 and 3). Furthermore, the study  
189

190 revealed antagonistic impact of N-PAH amendments in the mineralisation of  $^{14}\text{C}$ -B[a]P in soil (see  
191 supplementary data).

192

#### 193 **4. Discussion**

194 This study was designed to see if a more polar and soluble aromatic (such as N-PAHs) could stimulate  
195 phenanthrene and benzo[a]pyrene mineralisation in soil by measuring the lag phases, rates of mineralisation  
196 and extents of mineralisation. However, the results revealed that the presence of N-PAHs negatively affected  
197 the rates and extents of  $^{14}\text{C}$ -Phen and  $^{14}\text{C}$ -B[a]P mineralisation in soil, over time. Inhibition can be seen in any  
198 of the following: (a) increases in the length of the lag phase; (b) diauxic lag phases; (c) decreases in the  
199 degradation rates, and (d) decreases in the extent of mineralisation.

200 It is clear from the data presented in this study that  $^{12/14}\text{C}$ -phenanthrene was mineralised in the soil  
201 following the amendment of  $10\text{ mg kg}^{-1}$  and  $100\text{ mg kg}^{-1}$  phenanthrene. This is evident from the shorter lag  
202 phases, which generally decreased with soil contact time, the rates and extents of mineralisation. This is in  
203 agreement with some studies (Reid et al., 2002; Rhodes et al., 2008; Couling et al., 2010; Towell et al., 2010;  
204 Menga et al., 2017; Li et al., 2017; Mnif et al., 2017) in which the authors reported observations on the  
205 following: molecular mass (Reid et al., 2010), pre-exposure (Rhodes et al., 2008), microbial population and  
206 diversity (Towell et al., 2010; Li et al., 2017; Mnif et al., 2017), and the ubiquitous nature of PAHs in the  
207 environment at very low concentrations (Couling et al., 2010). Speedy mineralisation of phenanthrene was also  
208 recorded by Yoon et al. (2002), who reported that phenanthrene showed faster and stronger catabolic potential.  
209 In addition, Anyanwu and Semple (2015b; 2016b) attributed phenanthrene loss in soil to microbial elimination  
210 of the original compound (biological degradation), sequestration or removal as a result of soil-contact time  
211 (ageing).

212 Furthermore, this present study showed significant decrease in the extents of phenanthrene  
213 mineralisation with increased ageing and similar observation was reported by Couling et al. (2010) after 84 d  
214 soil contact time. The authors (Couling et al., 2010) attributed the decreases to amount of PAHs remaining in  
215 soil and decrease in chemical bioavailability to degrading soil microbial populations. However, sorption of  
216 phenanthrene to soil matrix, thereby reducing the bioavailability of the aromatic compound may also be  
217 important (Semple et al., 2007; Anyanwu and Semple 2016a, b).



218 It is widely known that the extent of degradation for different aromatic hydrocarbons are known to  
219 differ as a result of physico-chemical parameters such as molecular size, chemical structure, hydrophobicity  
220 and solubility (Stroud et al., 2007; Zhu et al., 2017; Doley et al., 2017). Studies have shown that structure and  
221 number of aromatic rings in combination with soil physico-chemical variables can affect the overall  
222 mineralisation kinetics of a particular compound. Thus, the >21 d lag phase observed with B[a]P in this present  
223 study is in agreement with the observations of Grosser et al. (1991) and Zhu et al. (2017). The authors noted  
224 that it took extensive lag phase of 3 – 4 weeks and 84 days before the mineralisation of B[a]P in soil and  
225 sediment, respectively. Also, studies on PAHs biodegradation in the soil environment have shown that  
226 degradation rates are inversely related to the number of aromatic rings (Cerniglia and Heitkamp, 1989; Eriksson  
227 et al., 2000). Thus, the <1% B[a]P mineralisation in this study is consistent with the findings of Atlas and Bragg  
228 (2015), who noted that contrarily to the low molecular PAHs, high molecular PAHs may not be degraded. This  
229 may be attributed to increase in size and angularity of B[a]P which resulted in a concomitant increase in  
230 hydrophobicity and electrochemical stability (Harvey, 1997).

231 The impact of a more polar and soluble heterocyclic aromatics (N-PAHs) was assessed on  
232 phenanthrene and benzo[a]pyrene mineralisation in soil. The study revealed that the presence of N-PAHs  
233 resulted in relatively increases in the length of lag phases. This confirms the report by Meyer and Steinhart  
234 (2000) and Willumsen et al. (2001) in heterocyclic compounds and azaarenes degradation, respectively. In  
235 support, Sutton et al. (1996) recorded 15 d lag phase in aerobic degradation of 4-methylquinoline.

236 Diauxic lag phase was displayed by B[h]Q despite reaching 5% mineralisation in this current study.  
237 This may be associated to the transient build-up of toxic metabolites and/or toxicity. Studies have shown that  
238 benzo-quinolines exert greater toxicity when they are bio-transformed to dihydrodiols, hydroxyls and epoxides  
239 (Warshawsky et al., 1992). In addition, large differences in N-PAHs physico-chemical properties, such as log  
240  $K_{ow}$ ,  $K_{oc}$ , variations in the type of N-substituents and/or ring position (Table 1) may influence mineralisation  
241 rates of the heterocyclic aromatics (Anyanwu and Semple, 2015b, c). However, the induction of catabolic  
242 enzymes by microbes to degrade B[h]Q chemical, may be attributed in this study.

243 When evaluating the environmental fate of organic contaminants, the extent of mineralisation is highly  
244 essential. The absence of positive statistically significance difference in the mineralisation of  $^{12/14}C$ -PAHs in  
245 the presence of N-PAHs in this current study, suggests inhibitory effect of N-PAHs on the biodegradation of  
246 PAHs in soil. This observation is in agreement with Meyer and Steinhart (2000) and Dyreborg et al. (1996a),

247 who reported inhibitions of hetero-PAHs (N-S-O) in the biodegradation of typical tar oil PAHs in soil and  
248 inhibitions of hetero-atoms in toluene degradation, respectively. Although not in the soil environment Lantz et  
249 al. (1997) also noted that heterocyclic analogues inhibited PAH degrading bacterium in culture media. In this  
250 study, N-PAHs impact could be attributed to their physico-chemical characteristics such as chemical structure  
251 (N-atoms), bioavailability, lower  $K_{ow}$ , solubility and/or chemical toxicity. This hypothesis is consistent with  
252 Millette et al. (1995), who suggested that the order of biodegradability of chemicals in complex mixtures is  
253 determined by their polarity, molecular size, structure and bioavailability. In addition, Anyanwu and Semple  
254 (2015b; 2016a, b) ascribed N-PAHs inhibitory effects to their recalcitrant fractions,  $pK_a$  values and/or N-atom  
255 position in the chemical structure.

256         Apart from the physico-chemical properties aforementioned, the impact of N-PAHs on the extent of  
257 phenanthrene and B[a]P mineralisation could also be attributed to microbial toxicity and/or inhibition of  
258 sensitive microbial populations (Demanèche et al., 2004; Anyanwu and Semple, 2015b; 2016a; Granato et al.,  
259 2017), indicating deterioration of ecosystems and the replacement of sensitive specie populations by resistant  
260 ones. This can have serious ecological consequences as well as impacts on the efficacy of bioremediation.

261         The significant impact ( $p < 0.05$ ) of 4,7-phenanthroline on  $^{14}C$ -phenanthrene mineralisation, may be  
262 related to the position of the N-atom on the aromatic structure. Yoon et al. (2002) observed that the rate of  
263 carbazole mineralisation significantly increased in phenanthrene-grown cell, linking it to similarities in  
264 chemical structure, and suggested the possibility that expression of genes encoding carbazole dioxygenase  
265 might be stimulated by phenanthrene. In addition, Lundstedt et al., 2003 reported that the heterocyclic  
266 compounds were almost degraded at similar rates to the PAHs with the same number of rings; however, they  
267 indicated that some N-heterocycles were degraded more slowly, for example, benzoquinoline.

268         In this current study, N-PAHs impacts were observed with increase in chemical concentration. The  
269 observed concentration related effect is in agreement with the findings of Willumsen et al. (2002) who reported  
270 inhibitory effect of chemical concentration on azaarene degradation; Sutton et al. (1996), who observed  
271 complete inhibition in the degradation of 4-methylquinoline by soil bacterium at  $172 \text{ mg L}^{-1}$ . In conformity,  
272 Anyanwu and Semple, 2015b; 2016a, b) reported concentration-specific impacts of N-heterocyclic aromatics  
273 (especially B[h]Q and 1,7-Phen) in soil, over time. Furthermore, the impact of N-PAHs on  $^{14}C$ -B[a]P  
274 mineralisation is consistent with the findings Meyer and Steinhart (2000), who reported that B[a]P was not  
275 influenced by the presence of hetero-PAHs until 111 d in the PAH / N-S-O soil amendments. However, the

276 <1% mineralisation of <sup>14</sup>C-B[a]P-N-PAH soils over time may be linked to N-PAHs antagonistic interaction  
277 and/or microbial toxicity (Demanèche et al., 2004; Anyanwu and Semple, 2015b; 2016a; Granato et al., 2017).  
278

## 279 **5. Conclusions**

280 The study was designed to see if the presence of 3-ring N-PAHs (a more polar and soluble heterocyclic  
281 aromatics) could enhance phenanthrene and B[a]P mineralisation in soil. However, inhibitory impact on <sup>14</sup>C-  
282 phenanthrene and <sup>14</sup>C-B[a]P mineralisation in soil by the presence 3 ring-N-PAHs was observed. The inhibitory  
283 impact resulted in increases in the length of lag phases, diauxic lag phases, decreases in the rates and extents of  
284 mineralisation. This suggests that losses of some PAH degradation capacity in polluted sites may be due to the  
285 presence of N-PAHs. Among the N-PAHs, B[h]Q displayed measurable impact on PAHs mineralisation  
286 processes, as shown in the study, indicating the greater toxicity of benzo-quinolines. This an important factor  
287 that must be considered in the development of sustainable bioremediation approaches for contaminated soils.

288

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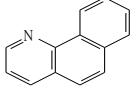
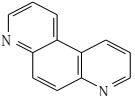
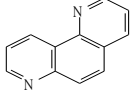
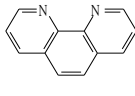
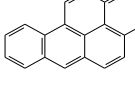
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435 **Table 1**

436 Physical and chemical properties of phenanthrene, N-PAH isomers and benzo[a]pyrene

Chemical	Chemical formula	Chemical structure	Molecular mass	Boiling point	Solubility 25°C (mg/L)	Vapour pressure 25°C (mm Hg)	Henry's law (atm·m <sup>3</sup> /mole)	Log K <sub>ow</sub>	Log K <sub>oc</sub>	Log K <sub>oa</sub> (m <sup>3</sup> /μg)
Phen	C <sub>14</sub> H <sub>10</sub>		178.2	340.00	1.15	1.21E-04	1.50E-05 <sup>a</sup>	4.46	4.32	9.12E-06 <sup>b</sup>
B[h]Q	C <sub>12</sub> H <sub>9</sub> N		179.2	339.00	78.70	2.08E-05	1.11E-06 <sup>a</sup>	3.43	4.32	0.001 <sup>b</sup>
4,7-Phen	C <sub>12</sub> H <sub>8</sub> N <sub>2</sub>		180.21	361.20	38.04	2.39E-05	1.85E-07 <sup>a</sup>	2.4	4.32	0.022 <sup>b</sup>
1,7-Phen	C <sub>12</sub> H <sub>8</sub> N <sub>2</sub>		180.21	365.10	30.64	2.39E-05	1.85E-07 <sup>a</sup>	2.51	4.32	0.022 <sup>b</sup>
1,10-Phen	C <sub>12</sub> H <sub>8</sub> N <sub>2</sub>		180.21	365.10	30.64	2.39E-05	1.85E-07 <sup>a</sup>	2.51	4.32	0.022 <sup>b</sup>
B[a]P	C <sub>20</sub> H <sub>12</sub>		252.31	377.00	0.17	2.44E-006	4.648E-006	5.99	5.40	7.823

437 <sup>a</sup> Henry's Law Constant (VP/WSol estimate using EPI values)438 <sup>b</sup> K<sub>p</sub> Octanol/air model (particle/gas partition coefficient).439 Source: [www.chemspider.com/Chemical-Structure](http://www.chemspider.com/Chemical-Structure)

440 **Table 2**441 Rates and extents of mineralisation of <sup>14</sup>C-phenanthrene and <sup>14</sup>C-benzo[a]pyrene in the presence of 10 mg kg<sup>-1</sup> N-PAHs during the 90 d incubation in soil

Parameters	Time (d)	Phen	1,10-Phen	1,7-Phen	4,7-Phen	B[h]Q	B[a]P	1,10-Phen	1,7-Phen	4,7-Phen	B[h]Q
Rates of mineralisation (% h <sup>-1</sup> )	1	0.87 ± 0.00	0.56 ± 0.10	0.38 ± 0.10	0.65 ± 0.30	0.90 ± 0.10	–	–	–	–	–
	30	0.42 ± 0.00	0.52 ± 0.20	0.74 ± 0.2	0.54 ± 0.00	0.27 ± 0.20	–	–	–	0.01 ± 0.00	–
	60	0.38 ± 0.00	0.17 ± 0.10	0.34 ± 0.10	0.77 ± 0.10	0.40 ± 0.00	–	–	–	–	–
	90	0.52 ± 0.00	0.49 ± 0.00	0.57 ± 0.10	0.62 ± 0.10	0.51 ± 0.00	–	–	–	–	–
Extents of mineralisation (%) (21 d)	1	43.39 ± 0.50*	38.66 ± 1.60	33.67 ± 6.50	41.92 ± 7.90	39.19 ± 1.20	0.05 ± 0.00	0.41 ± 0.10	0.03 ± 0.00	0.82 ± 0.20	–
	30	41.08 ± 0.70*	42.36 ± 5.10	46.46 ± 0.20	49.99 ± 0.20	37.95 ± 5.30	0.78 ± 0.00	0.10 ± 0.10	0.06 ± 0.00	0.64 ± 0.00	0.14 ± 0.00
	60	28.94 ± 0.20*	24.89 ± 0.90	28.04 ± 1.50	33.84 ± 0.10	22.19 ± 1.70	0.11 ± 0.00	0.11 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.01 ± 0.00
	90	29.60 ± 0.10*	27.41 ± 1.70	30.77 ± 0.60	31.76 ± 0.00	31.04 ± 0.20	0.13 ± 0.00	–	0.02 ± 0.00	–	0.12 ± 0.00

442 Conc = concentration (mg kg<sup>-1</sup>), (–) = no mineralisation, data = amended compounds, \* = p<0.05.

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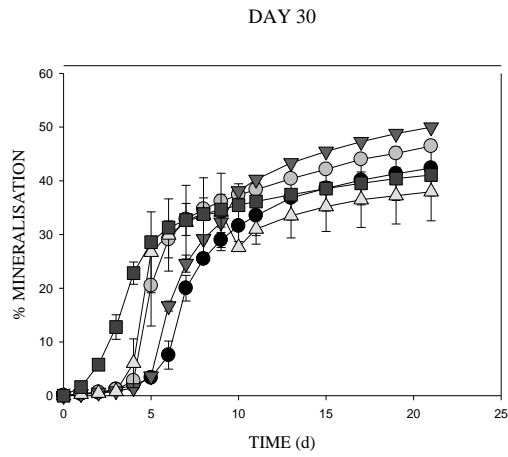
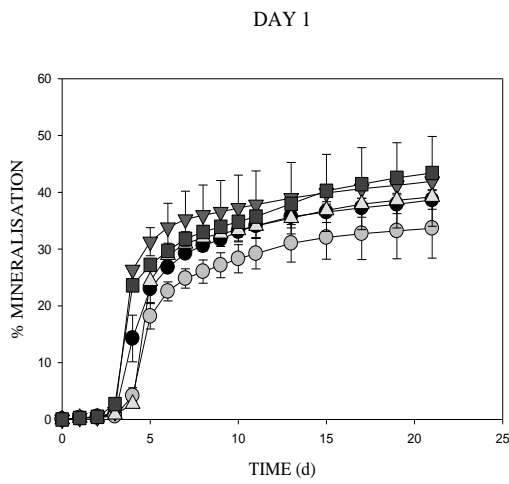
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447 **Table 3**448 Rates and extents of mineralisation of <sup>14</sup>C-phenanthrene and <sup>14</sup>C-benzo[a]pyrene in the presence of 100 mg kg<sup>-1</sup> N-PAHs during the 90 d incubation in soil

Parameters	Time (d)	Phen	1,10-Phen	1,7-Phen	4,7-Phen	B[h]Q	B[a]P	1,10-Phen	1,7-Phen	4,7-Phen	B[h]Q
Rates of mineralisation (% h <sup>-1</sup> )	1	0.75 ± 0.30	0.49 ± 0.40	0.73 ± 0.10	0.77 ± 0.10	0.48 ± 0.40	–	–	0.01 ± 0.00	–	0.01 ± 0.00
	30	0.67 ± 0.00	0.25 ± 0.10	0.47 ± 0.00	0.26 ± 0.10	0.49 ± 0.00	–	0.01 ± 0.00	–	–	–
	60	0.57 ± 0.00	0.28 ± 0.10	0.40 ± 0.10	0.36 ± 0.00	0.32 ± 0.20	–	–	–	–	–
	90	0.47 ± 0.00	0.52 ± 0.00	0.62 ± 0.00	0.61 ± 0.10	0.56 ± 0.10	–	–	–	–	–
Extents of mineralisation (%) (21 d)	1	38.36 ± 5.30*	33.73 ± 4.30	43.16 ± 9.20	37.39 ± 0.90*	50.77 ± 4.40	0.09 ± 0.10	–	–	0.02 ± 0.00	0.16 ± 0.00
	30	41.51 ± 3.60*	30.91 ± 2.20	45.33 ± 0.30	42.37 ± 3.30*	45.80 ± 1.90	0.02 ± 0.00	0.88 ± 0.20	0.18 ± 0.00	0.23 ± 0.00	0.20 ± 0.00
	60	25.61 ± 1.10*	20.81 ± 5.20	33.51 ± 0.00	26.38 ± 0.30*	39.43 ± 3.90	0.06 ± 0.00	0.01 ± 0.00	0.19 ± 0.10	0.08 ± 0.00	0.06 ± 0.00
	90	31.40 ± 0.30*	35.03 ± 0.70	36.08 ± 0.80	32.32 ± 1.10*	40.69 ± 0.60	0.12 ± 0.00	0.01 ± 0.00	0.09 ± 0.00	0.15 ± 0.00	0.06 ± 0.00

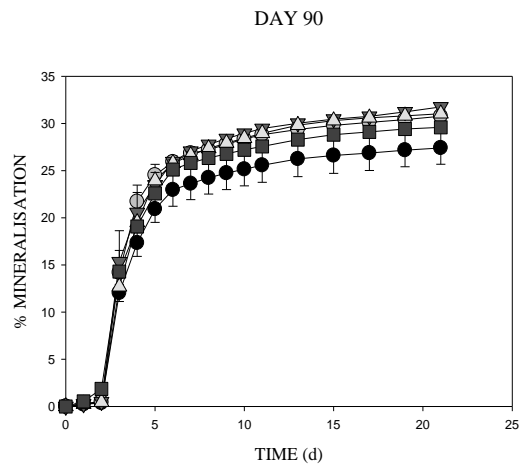
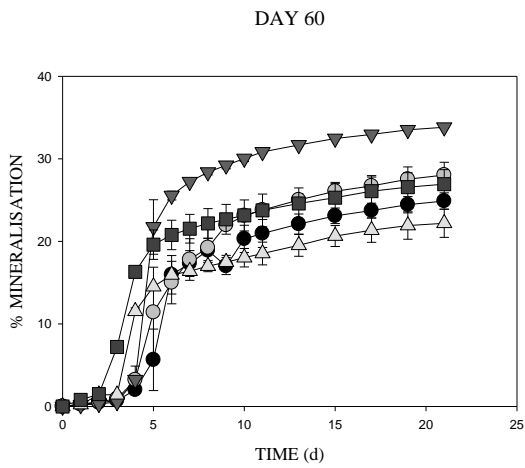
449 Conc = concentration (mg kg<sup>-1</sup>), (–) = no mineralisation, data = amended compounds, \* = p<0.05

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454 **Fig. 1.** Mineralisation of  $^{12/14}\text{C}$ -phenanthrene in the presence of  $10 \text{ mg kg}^{-1}$  N-PAH amended soils during the  
455 90 d incubation. The 1 – 90 d graph shows: 1,10-Phen (●), 1,7-Phen (○), 4,7-Phen (▼), B[h]Q (Δ) and Phen  
456 (■).

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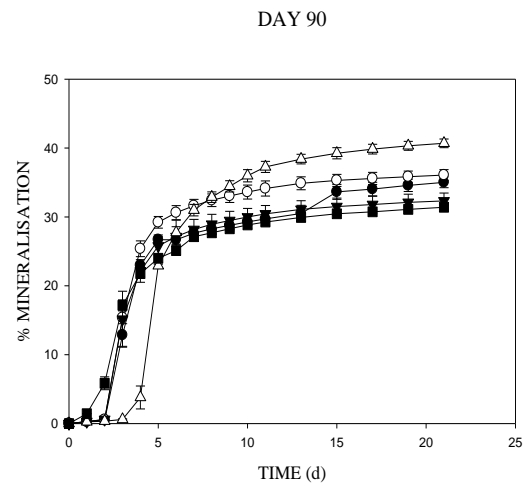
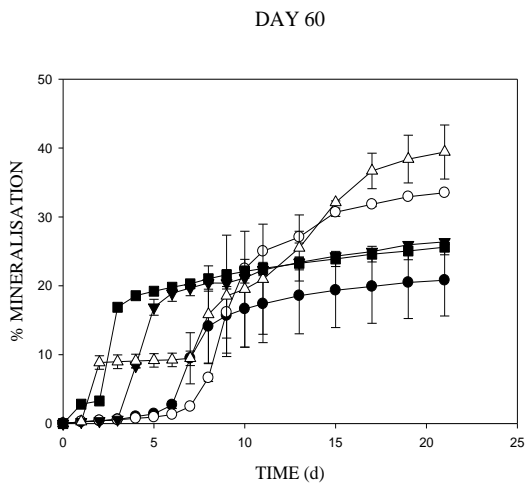
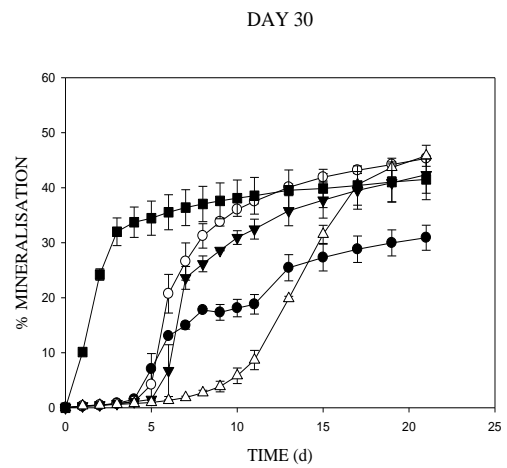
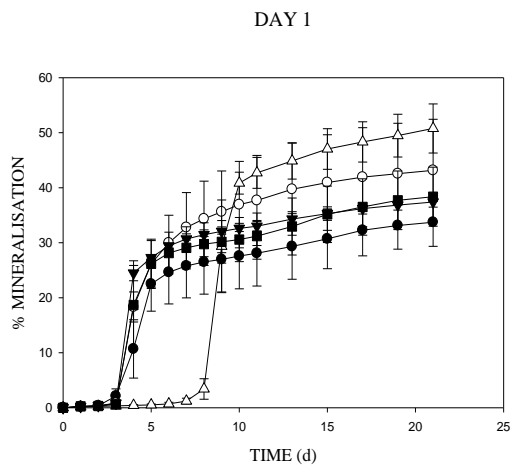
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467 **Fig. 2.** Mineralisation of  $^{12/14}\text{C}$ -phenanthrene in the presence of  $100 \text{ mg kg}^{-1}$  N-PAH amended soils during the  
 468 90 d incubation. The 1 – 90 d graph shows: 1,10-Phen (●), 1,7-Phen (○), 4,7-Phen (▼), B[h]Q (Δ) and Phen  
 469 (■).