Effects of pre-exposure on the indigenous biodegradation of ^{14}C-phenanthrene in Antarctic soils

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Abstract

The aim of this study was to investigate the biodegradation of phenanthrene in five Antarctic soils over 150 days at various temperatures and under slurry conditions. The development of catabolic activity was measured over time (1, 30, 60, 150 days) by the addition of ^{14}C-phenanthrene and measuring changes in the lag phases, rates and extents of ^{14}C-phenanthrene degradation. As the temperature increased (4 ºC, 12 ºC, 22 ºC, 22 ºC slurry), the highest extents of ^{14}C-phenanthrene mineralisation increased significantly (0.46%, 12.21%, 24.82%, 60.81%), respectively. This was due to changes in the water availability and ^{14}C-phenanthrene dissolution in aqueous phase, thus enhancing bioaccessibility of the contaminant to indigenous microorganisms within the soil. High catabolic activities can develop in Antarctic soils where appropriate conditions are ensured. However, further studies are however needed to explore the changes in microbial community structure that occur at different incubation temperatures.

Keywords: Antarctica, pre-exposure, biodegradation, ^{14}C-phenanthrene

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are an important class of environmental pollutants (Doick et al., 2003). Their potential for long range atmospheric transport (Prevedouros et al., 2004), ubiquitous presence in the atmosphere (Garrido et al., 2014), marine (Latimer and Zheng, 2003) and soil environments (Wilcke, 2007), low aqueous solubility, high octanol water coefficient (log K_{ow}) and adverse health effects (Kim et al., 2013) has fuelled research interest into their sources and fate in varying environments. The fate of PAHs in the soil environment is critical to the amount of PAHs present in the total environment. This is because about 90% of the global environmental PAHs are stored in soils (Wild and Jones, 1995; Agarwal et al., 2009). Despite this, soils can serve as a disturbing source of PAHs to the atmosphere (Cousins and Jones, 1998; Wang et al., 2010). As a result, PAH concentrations in soils have been found to correspond to concentrations in the atmospheric environment (Zhao et al., 2015).

PAHs are removed from soil primarily through microbial activity as these aromatics represent sources of carbon and energy for microbial metabolism (Semple et al., 2006; Couling et al., 2010; Guo et al., 2010). As a result, PAH-degrading microorganisms have been isolated from many different soils, including tropical (Obayori et al., 2008; Obayori et al., 2009; Guo et al., 2010; Isaac et al., 2013), temperate (Johnsen et al., 2006; Ogbonnaya et al., 2014a) and extreme temperature environments, such as cold (Baraniecki et al., 2002) and hot deserts (Abed et al., 2015a; 2015b). In order for microbial degradation of PAHs to occur, the presence of microorganisms with the appropriate genetic potential is essential (Peng et al., 2008). The microorganisms must be in the same environment as the PAH and the PAH must be able to be physically transferred to the site of metabolism in the microorganism (Macleod et al., 2001). Bioavailability is also important and depends on the physicochemical properties and concentration of the PAHs (Guo et al., 2010; Sayara et al., 2010), the properties of the

soil (mainly organic matter content, moisture content/water activity and temperature), microorganisms present (Semple et al., 2007; Ogbonnaya et al., 2014a; 2016), length PAH-soil contact time (Leonardi et al., 2007; Rhodes et al. 2010; Ogbonnaya et al., 2014a) and presence of co-substrate (Sayara et al., 2011).

So, how do microorganisms develop the ability to degrade PAHs? Prior exposure of soil microorganisms to PAHs or similar chemicals from either natural or anthropogenic sources is believed to be important to the development of PAH degradation ability in microorganisms (Johnsen and Karlson, 2005; Couling et al., 2010). Microbial development or adaptation is controlled by the amount of the PAH in contact with the microorganism and the length of time of the contact (Bosma et al., 1996; Macleod, et al., 2001; Couling et al., 2010). Where no prior exposure to a PAH has occurred, the microorganisms would require genetic alterations (Semple et al., 2003), which may result in new metabolic capabilities enabling the microorganisms to degrade PAHs (van der Meer et al., 1992).

The Antarctic environment is still considered one of the Earth's last pristine environments (Anderson et al., 2006). Although increased human activities in the form of tourism and the establishment of scientific bases in the region has led to hydrocarbon contamination of some soils (Coulon et al., 2005), PAHs are either undetectable (Aislabie et al., 1999), at pre-industrial (Wilcke, 2000) or background levels (Johnsen and Karlson, 2005). Antarctic soils have been described as cold desert soils (Bockheim, 1997) and are characterised by extremely low temperatures, low biological activity, low presence of nutrients, poor moisture and low organic matter contents (Campbell and Claridge, 2009). The unique and extreme properties of Antarctic soils, in addition to their "pristine" nature make the question of the development of PAH catabolic activity in Antarctic soils an interesting one (Okere et al., 2012a). To the best of the authors' knowledge, little or no work has focused on the effect(s) of pre-exposure and increasing contact time of indigenous Antarctic soil microflora to PAHs and the

biodegradation of PAHs. Therefore, the aim of this study was to investigate the effect(s) of exposing five Antarctic soils to ^{12}C-phenanthrene over 150 days at different temperature conditions on the development of ^{14}C-phenanthrene catabolism in the soils.

2. Materials and methods

2.1 *Materials*

Phenanthrene (>99.6%), and [9-^{14}C] phenanthrene (specific activity = 50 mCi mmol^{-1}, radiochemical purity >95%) standards were obtained from Sigma Aldrich, UK. Chemicals for the minimal basal salts (MBS) solution were obtained from BDH Laboratory Supplies and Fisher Chemicals. The liquid scintillation cocktail (Ultima Gold) and 7 ml glass scintillation vials were obtained from Canberra Packard, UK. Sodium hydroxide was obtained from Sigma Aldrich, UK. Dichloromethane, hexane and methanol were supplied by Merck, Darmstad, Germany. Agar and plate count agar were obtained from Oxoid Ltd, UK.

2.2 *Soils sampling and bulk characterization*

Composite topsoil (0-5 cm) samples (5) were collected using a stainless-steel corer from different locations of Livingstone Island, Antarctica and labelled A – E. According to sample transportation standards, the samples were frozen (-20 ^{o}C) in sterile glass jars and then transported to Lancaster Environment Centre. The soils were allowed to defrost and further air-dried and passed through a 2 mm sieve to remove stones and fibrous material. Then the soils were subject to physical and chemical analysis to determine their properties (Table 1). Soil redox, soil pH and soil moisture content were measured by standard methods according to Cabrerizo et al. (2011). Particle size analysis and calculations were determined according to the method by Gee and Bauder (1979) and Gee and Bauder (1986), respectively. Total

121 carbon and nitrogen were determined by using a Carlo Erba CHNS-OEA 1108 CN-Elemental
122 analyser after oven drying (105 ºC) 4 mg of and sieved (2 mm) soil samples. Total organic
123 carbon (TOC) was determined after heating soils to 430 ºC removing all organic carbon,
124 measuring the ash containing inorganic carbon alone and the TOC determined by mass
125 balance (Rhodes et al., 2007) .

126

127 2.3 *PAH concentrations in soil*

128 For extraction and quantification, 30 g of soil samples were homogenized, dried using
129 anhydrous sodium sulfate and ground using a mortar and a pestle. The samples were
130 transferred into a soxhlet cellulose thimble (Whatman) and extracted in soxhlet apparatus
131 over 24 h, using dichloromethane:methanol (2:1 v/v). Samples were spiked with per-
132 deuterated PAHs standards (anthracene-d10, crysene-d12 and perylene-d12) prior extraction.
133 Extracts were further reduced in a rotary evaporator to 1 ml and then solvent-exchanged into
134 isooctane. Samples were then fractionated on a 3 % deactivated alumina column (3 g) with a
135 top layer of anhydrous sodium sulfate, where each column was eluted with 12 ml of
136 dichloromethane:hexane (2:1 v/v). PAH fractions were further concentrated in a rotary
137 evaporator and solvent-exchanged to isooctane under a gentle stream of nitrogen. All the
138 samples were analysed by GC-MS using a Thermo Electron (San Jose, CA, USA; model
139 Trace 2000 operating in selected ion monitoring (SIM) mode (Okere et al., 2012a). Details of
140 temperature programs and monitored ions are given elsewhere (Cabrerizo et al., 2009;
141 Cabrerizo, et al., 2011).

142

143 2.4 *Quality Assurance/Control*

144 Strict quality assurance and control measures were implemented during sampling,
145 transportation and analysis. During analysis, field and laboratory blanks were introduced after
146 every three (3) soil samples. Field blanks as they were prepared at the sampling sites to
147 determine the potential for contamination of the samples by PAHs not associated with the soil
148 samples, phenanthrene, fluoranthene and pyrene concentrations accounted for less than 3% of
149 the total PAH content in the sample. Samples therefore were not blank corrected. The
150 surrogate percent recoveries from the soil samples reported here were (mean ± SD): 70% ±
151 11; 105% ± 17 and 90% ± 13 for phenanthrene-d10, chrysene-d12 and perylene-d12,
152 respectively.

153

154 2.5 *Soil spiking and exposure to ^{12}C-phenanthrene*

155 To expose soils to ^{12}C-phenanthrene, soils were spiked with ^{12}C-phenanthrene following the
156 method recommended by Doick et al. (2003). ^{12}C-Phenanthrene standards were prepared in
157 toluene (7.5 ml per 250 g soil) to deliver a concentration of 50 mg kg^{-1}, where an initial 50 g
158 of the soil was spiked in the mixing vessel (stainless-steel spoon) and blended for a minute
159 and the remainder 200 g soil was added in 100 g aliquots and blended for 5 minutes. Toluene
160 was allowed to volatilise after mixing with the initial 50 g to prevent damage to microbial
161 cells in soils. Blank soils which were not not spiked with ^{12}C-phenanthrene were also
162 prepared to account for background ^{14}C-associated activity. All the soils were then contained
163 in sealed amber glass jars and left incubated in the dark at 4 ºC , 12 ºC and 22 ºC for 1, 30,
164 60 and 150 days.

165

166 2.6 *Catabolism of ^{14}C-phenanthrene in soil*

167 The catabolic activity of ^{14}C-phenanthrene by indigenous microflora in the soils was
168 determined in 250 ml screw-cap Erlenmeyer flasks (respirometers) (Reid et al., 2001) after 1,

30, 60 and 150 days contact times. Pre-exposed soils (10 g) rehydrated to 40-60% water holding capacity were placed in a respirometer and spiked with ^{12}C- (>99.6%) and ^{14}C-phenanthrene (80 Bq ^{14}C-phenanthrene g^{-1} soil) using toluene as a carrier solvent. A 7 ml scintillation vial containing 1 M NaOH was attached to the screw cap to serve as a CO_2 trap. The respirometers were stored in the dark at the respective temperatures which the soils were exposed (4 ºC, 12 ºC, 22 ºC). A slurry system was also set up containing 30 ml mineral basal salts (MBS) medium as Ogbonnaya et al. (2014b) and placed on a SANYO® Gallenkamp orbital incubator set at 100 rpm and 22 °C to agitate and ensure adequate mixing over the period of the incubation. NaOH traps were replaced every 24 h, after which 6 ml of Ultima Gold scintillation cocktail was added to each spent trap and the contents analysed on a Packard Canberra Tri-Carb 2250CA liquid scintillation counter. The incubation lasted for 21 days. Lag phases were measured as the time (days) before ^{14}C-phenanthrene mineralisation reached 5%. Analytical blanks containing no ^{14}C-phenanthrene were used for the determination of levels of background radioactivity.

2.7 *Statistical analysis*

Respirometric assays were analysed in triplicate and error bars presents standard error mean for n=3. SIGMA STAT version 2.03 software package was used for the analysis of the data. The significance of ^{14}C-phenanthrene degradation between soils and temperatures were assessed by implementing ANOVA and Tukey's tests.

3.0 **Results**

3.1 *Soil physico-chemical properties*

192 The physico-chemical properties of the five selected soils from Livingstone Island were
193 similar. They were found to be consistent with properties of Antarctic soils published
194 elsewhere (Campbell and Claridge, 2009; Okere et al., 2012b). All the soils were dominantly
195 sandy (≥ 88%) in nature, with little or no silt content (0-4%). All the soils were slightly
196 alkaline and characterised by very low TOC (< 0.5%), moisture (< 1.5%) and N contents (≤
197 0.26%) (Table 1). However, all soils exhibited neutral and slightly alkaline pH (6.7 – 7.9)
198 conditions favourable for microbial growth. Similarly, the PAH levels were shown to be very
199 low with highest total PAH concentration in soil E (0.85 mg kg^{-1}) and lowest concentration in
200 soil C (0.28 mg kg^{-1}). Low molecular weight PAHs such as phenanthrene, fluoranthene,
201 anthracene and pyrene were found in all soils, whilst benzo (a) pyrene was found in only soils
202 A and D (Table 1).

203

204 3.2 *Catabolism of ^{14}C-phenanthrene in pre-exposed soils at different temperature regimes*

205 The mineralisation of ^{14}C-phenanthrene was measured in soils at different temperatures and
206 conditions (4 ºC, 12 ºC, 22 ºC and slurry) with increasing soil-phenanthrene contact time (1,
207 30, 60, 150 days). The effects of temperature, biodegradation condition and contact time on
208 lag phase, fastest rate (per day) and extent of ^{14}C-phenanthrene mineralisation were observed.

209 When assays were incubated at 4 ºC, there was no observed lag phase across all contact times
210 and the maximum rate of ^{14}C-phenanthrene mineralisation did not exceed 0.06% d^{-1} which
211 was often observed during first day of respirometry assay and observed in the 1 day contact
212 time (Table 2). There was statistically insignificant difference ($P > 0.05$) in the maximum
213 rates of ^{14}C-phenanthrene mineralised in all five soils at all contact times (Table 3). The
214 highest extent of ^{14}C-phenanthrene mineralised was 0.46% (soil B) and the lowest was 0.23%
215 (soil E) during 1 day contact time (Figure 5). Increasing the contact time to 30, 60 and 150

days did not result in any significant change ($P > 0.05$) in the extent of ^{14}C-phenanthrene mineralisation (Table 4).

At a higher temperature (12 ºC), the lag phase was not observed until after 60 days contact time in soils C (14.87 days) and D (4.76 days), where the lag phase of soil D was statistically the shortest ($P < 0.001$) compared to other soils under 12 ºC assay condition (Table 2). However, as the contact time increased to 150 days, the lag phase in soil D statistically increased ($P < 0.001$) following further exposure to 12.91 days which was statistically similar to other soil C (Table 2). Similar to 4 ºC soil assay, the maximum rate of ^{14}C-phenanthrene in 12 ºC soil did not exceed 0.07% d^{-1} following 1 and 30 days contact times, but after pre-exposure at 60 days, maximum rate of mineralisation significantly increased to 0.74%, 1.76% and 0.20% d^{-1} in soils C, D and E, respectively (Table 3). Further increase in contact time did not sustain the rates of mineralisation, rather led to decreased rates, except for soil C (Table 3). The extents of ^{14}C-Phenanthrene mineralisation after 1 d contact time were similar in all five soils (Figures 1-5, Table 2). After 30 days contact time, the extents of ^{14}C-phenanthrene mineralisation in all the soils decreased, but increasing contact time to 60 days resulted in significant increases ($P < 0.05$) in ^{14}C-phenanthrene mineralisation in soils C (5.3%) and D (12.2%) alone. This was maintained after the 150 days pre-exposure in soils C and D (Table 4).

At 22 ºC, lag phases were observed and they increased in soils D and E as contact time increased but there was statistical increase ($P < 0.05$) only at 150 days contact time compared to other time points in both soils. Lag phase insignificantly decreased ($P = 0.49$) in soil C as contact time increased from 60 to 150 days (Table 2). Maximum rates of ^{14}C-phenanthrene mineralisation increased ($P < 0.05$) with contact time in soils C, D and E compared to the shorter contact times (1 and 30 days) (Table 3). Microbial catabolic activity in soil D exhibited the highest maximum rate of ^{14}C-phenanthrene mineralisation (10.6%) compared to

241 all other soils and contact times. The highest extent of ^{14}C-phenanthrene mineralisation in all
242 the soils after 1 d contact time was in soil B (2.17%) (Table 4). Exposing the soils to ^{12}C-
243 phenanthrene for additional 30 days led to no significant changes ($P > 0.05$) even though
244 there were decreases in extents of mineralisation in soils A, B and C. However, after 60 days
245 contact time, ^{14}C-phenanthrene mineralisation in soils C, D and E increased significantly ($P <$
246 0.05) to 6.7%, 16.8% and 19.0%, respectively. A further significant increase was observed in
247 soil D (24.8%) but significantly decreased in soil E (8.3%) after 150 days contact time (Table
248 2).

249 Under the 22 ºC slurry conditions, lag phase, maximum rates and extent of ^{14}C-phenanthrene
250 mineralisation were much more obvious. For instance, it was only under the slurry condition
251 that all contact time points recorded lag phases, which differed in time and soil type. At 1 day
252 contact time, soil A recorded the shortest (5.6 days) lag phase ($P < 0.05$) compared to other
253 soils but as contact time increased to 30 days, soil A had the longest lag phase, whilst soil E
254 had the shortest (2.3 d) lag phase ($P < 0.001$) (Table 2) (Figure 5; Table 2). Concerning
255 maximum rates, microorganisms in soil E consistently showed highest rates of ^{14}C-
256 phenanthrene mineralisation ($P < 0.001$) compared to all other soils and across all contact
257 times, except 60 days contact time where soil C had fastest rate (25.7% d^{-1}). As the contact
258 time increased, maximum rates of mineralisation in soil E increased to 27.3% d^{-1} and then
259 was stable at 19.9% d^{-1} and 21.0% d^{-1} at 30, 60 and 150 days contact times, respectively
260 (Table 3) which were significantly higher ($P < 0.05$) compared to other soils. Unsurprising,
261 the highest extent of ^{14}C-phenanthrene mineralisation was also in soil E (60.8%) at 1 day
262 contact time, which was significantly higher ($P < 0.001$) than extents of mineralisation
263 amongst other soils (A, B, C, D). Soil E consistently had highest extent of mineralisation
264 across all contact times, except at 150 days contact time where soil D had 38.8%, which was
265 significantly ($P = 0.009$) higher than soil E. Soils A-C had insignificant change in extents of

mineralisation until 150 days contact time, where there were significant reductions ($P < 0.05$) (Table 4).

4.0 **Discussion**

4.1 *Soil physico-chemical properties*

As in previous studies, soils collected from Livingstone Island of Antarctica Island, distant from persistent human activities were characterised by extremely low nutrient, organic carbon and moisture conditions (Campbell and Claridge, 1987; Okere et al., 2012b). Losses of these components are common with coarse sandy soils, clays are minor in such soils due to the dominance of physical weathering processes over chemical weathering (Egli et al., 2008; Spinola et al., 2017). Apparently, vegetative cover and biological presence were found to be limited within the studied region, which further contributed to the low level organic carbon content, but does not rule out microbial presence (Okere et al., 2012b). The soil organisms encounter extremely low water and nutrient content, very low temperatures with ice formations, freeze–thaw cycles, prolonged darkness in winter and short summer spells (Cary et al., 2010). Despite the harsh environmental conditions, the alkaline pH condition is favourable for bacterial growth and activities (Aislabie et al., 2001; Baraniecki et al., 2002; Okere et al., 2012b). PAHs were found in all the five soils at levels (very low) similar to those reported in uncontaminated/pristine soils (Johnsen and Karlson, 2005; Cabrerizo et al., 2012; Okere et al., 2012b).

4.2 *Effects of soil properties on bioaccessibility*

288 Firstly, due to the nature of Antarctic soils (sandy, low TOC, nutrients, moisture content and
289 PAH concentrations) (Table 1), the bioavailability and bioaccessibility of the ^{12}C-
290 phenanthrene spiked into the soils was not expected to be reduced by adsorption to either soil
291 organic matter (SOM) or soil mineral components as soil-^{12}C-phenanthrene contact time
292 increased. In low organic matter sandy soils with < 4% moisture content, the retention of
293 hydrophobic organic contaminants (HOCs), like PAHs, is controlled by their adsorption onto
294 mineral surfaces rather than onto soil organic matter (Qu et al., 2008; Zhang et al., 2011).
295 Indeed, strong interactions between the low SOM fractions and mineral surfaces occur to
296 create condensed domains that can support PAH adsorption (Wang and Xing, 2005; Wang et
297 al., 2005). Theoretically, this means reduced bioaccessibility of the ^{12}C-phenanthrene due to
298 sequestration to either soil organic matter or soil mineral components, but they were not the
299 only limiting factors to the adaptation of the indigenous microbes to ^{14}C-phenanthrene
300 mineralisation in these Antarctic soils. The other factors that must have contributed to low
301 bioaccessibility of phenanthrene were low moisture conditions and temperature for microbial
302 catabolic activities in all soils.

303

304 4.3 *Catabolism of ^{14}C-phenanthrene in pre-exposed soils under different temperature*
305 *regimes*

306 The effects of exposing the indigenous microbes to ^{12}C-phenanthrene on their ability to
307 mineralise ^{14}C-phenanthrene were increased as exposure and incubation temperatures
308 increased (Figure 1). Exposure and incubation at 4 ºC had no significant effect on either the
309 rates or extents of ^{14}C-phenanthrene mineralisation in all five soils studied. Less than 1% of
310 the ^{14}C-phenanthrene was mineralised (no lag phase) throughout the 150 day exposure period
311 and the rates of ^{14}C-phenanthrene mineralisation remained less than 0.06 % d^{-1} over the same

312 period (Table 3). Research by Ogbonnaya et al. (2014a) and Oyelami et al. (2015) showed
313 that it would require high concentrations of biochar and activated carbon (super sorbents),
314 respectively after prolonged soil-PAH contact time (>100 days) to drastically lower
315 phenanthrene mineralisation below 10%. In this study, phenanthrene mineralisation at 4 ºC
316 did not exceed 1%, which was contrary to the levels of phenanthrene mineralisation in soils
317 sourced from Antarctica having different soil properties but under similar conditions in
318 Coulon et al. (2005). This study thus reiterates the important role of temperature on microbial
319 biodegradation of PAHs in soils. Temperature is important because it influences the rates and
320 extents of PAH degradation in soils in a number of ways. Firstly, microbial activity obeys the
321 Arrhenius relationship because it increases with increasing temperature (Leahy and Colwell,
322 1990), which usually doubles for each 10 ºC rise in temperature (Bossert and Bartha, 1984;
323 Coulon, et al., 2005). Microbial activities by psychrophiles and psychrotrophs are expected in
324 Arctic and Antarctic environments but the catabolic activities were not observed in
325 phenanthrene mineralisation despite pre-exposure probably due to temperature-dependent
326 biochemical activities of phenanthrene degraders in the soils (D'Amico et al., 2006).
327 Secondly, microorganisms are only able to degrade chemicals that have been dissolved in the
328 aqueous phase (Semple, et al., 2003). At 4 ºC, PAHs are more viscous, less volatile and less
329 soluble, therefore impeding bioaccessibility and diffusion rates to microorganisms, hence,
330 only minute fractions of PAHs if any will be in the aqueous state (Margesin and Schinner,
331 2001). Also, any moisture present in the soil pore spaces will be frozen at 4 ºC (low liquid
332 water availability), making it difficult for the ^{12}C-phenanthrene to be accessed by the
333 microbes for adaptation.

334 As the exposure time and incubation temperature was increased from 4 ºC and 22 ºC, there
335 were noticeable changes in the catabolic activity of the indigenous microorganisms in some
336 of the soils (Tables, 2, 3, 4). More precisely, there were increases in the extents of ^{14}C-

phenanthrene mineralisation as the temperature increased to 22 ºC, due to increased water availability and phenanthrene dissolution in aqueous phase, thus enhancing bioaccessibility of the contaminants to indigenous microorganisms (ten Hulscher and Cornelissen, 1996; Coulon et al., 2005). In respect to catabolic activities, lag phases were observed in soils C and D at 12 ºC after 60 to 150 days contact times and under the 22 ºC (soils C, D, E) incubation conditions. This accurately coincided with extents of ^{14}C-phenanthrene mineralisation in the said soils, where rates of mineralisation exceeded 1% d^{-1}, extents of mineralisation exceeded 5%. Despite all the soils in this study being collected under the same climatic conditions (Antarctica) and exhibited similar physico-chemical properties (N, TOC, pH, texture, moisture content) which were invariably low, catabolic activities varied with contact time and temperature. Although not investigated, this suggests that the different soils contained different spectra and density of psychrophilic and psychrotrophic microorganisms capable of degrading phenanthrene (Eriksson et al., 2003; Antizar-Ladislao et al., 2008).

Catabolic activities were mainly pronounced under slurry conditions at 22 ºC. The lag phases in each soil showed a decreasing trend as the incubation time increased until 60 days contact time indicating an adaptation of the indigenous microorganisms to the presence of phenanthrene (Couling et al., 2010; Ogbonnaya et al., 2014b; Oyelami et al., 2015). Microbial adaptation would have been through increase in microbial population (growth of mesophiles), catabolic enzyme induction and transgenic manipulations degrading populations (Top and Springael, 2003; Ogbonnaya 2014b). Microbial adaptations followed a sigmoidal pattern and the period took much longer time (60 days) to be below 5 days compared to adaptation period of phenanthrene mineralisation in UK soils (Couling et al., 2010; Rhodes et al., 2010; Ogbonnaya et al., 2014b) despite having lower organic carbon content and being under similar conditions (slurry). Microbial diversity, activities and transgenic manipulations

362 may be much higher in UK soils compared to pristine soils of Antarctica owing to initial
363 environmental stressors within the sample sites such as temperatures, freeze-thaw cycles, low
364 organic carbon and unavailability of moisture (Pointing et al., 2009; Rao et al., 2012; Cowan
365 et al., 2014). It also happens that the catabolic activity amongst the Antarctica soils differed,
366 where soil E exhibited the highest rate and extent of ^{14}C-phenanthrene mineralisation, as well
367 as the shortest lag phases compared to other soils after 1-60 days contact time. Soil E already
368 had catabolic potential via constitutive or actively induced enzymes right from the onset.
369 Also, soil E had higher bioaccessibility due to non-detectable organic carbon and the highest
370 concentration of PAHs (Table 1) and much higher phenanthrene (0.32 mg kg^{-1}) prior spiking
371 (Couling et al., 2010; Rhodes et al., 2010; Ogbonnaya et al., 2014a). When compared with
372 previous studies, the catabolic activity recorded in the Antarctic soil E was higher than that
373 observed in Couling et al. (2010), where less than 60% of 75 mg kg^{-1} ^{14}C-phenanthrene
374 spiked was mineralised by indigenous microorganisms in a 2.7% TOC soil from the UK at 1
375 day contact time. Also, Rhodes et al. (2010) observed over 60% of 10 mg kg^{-1} ^{14}C-
376 phenanthrene mineralisation in a 1.7% TOC control soil in UK at 1 day contact time.
377 Although, soil E had a non-detected TOC, it showed that such 'pristine' soils inhibit catabolic
378 potentials and when supported, mineralisation by indigenous microorganisms can be
379 enhanced.
380
381 This study further supports Coulon et al. (2005) in that high catabolic potential can be
382 observed in Antarctic soils when right conditions are appropriate. Slurrying the system
383 ensured there was more ^{14}C-phenanthrene in solution and agitation ensured maximum contact
384 between the microorganisms and the substrate. Doick and Semple (2003) practically showed
385 that soil slurrying enhances soil surface area, thus facilitating partitioning of phenanthrene
386 into the aqueous mixture where microbial mobility would have remarkably increased. A

general trend marked by a static extent of ^{14}C-phenanthrene mineralisation soils with increasing exposure time to ^{12}C-phenanthrene was observed from day 1 to day 60.

5.0 **Conclusion**

Antarctic soils are peculiar because of their unique soil characteristics and "pristine" nature. This study investigated the effect of exposing five Antarctic soils to ^{12}C-phenanthrene at different temperatures and assay conditions on the rates and extents of indigenous biodegradation of ^{14}C-phenanthrene. Our findings suggest that exposure and incubation temperature are important limiting factors for the adaptation of indigenous Antarctic soil microorganisms to ^{14}C-phenanthrene biodegradation. Further studies with other Antarctic soils and PAHs are needed to verify this claim as well as also identify what specific changes are occurring in the soil microbial communities as exposure time to PAHs increase.

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Table 1 Physical and chemical properties of five soils from Livingstone Island, Antarctica. (ND - Not Detected)

PAH (ng g^{-1}dry wt soil)	**Soil A**	**Soil B**	**Soil C**	**Soil D**	**Soil E**
Methylphenanthrene	ND	0.05	0.05	ND	0.08
Dibenzothiophene	0.14	ND	ND	ND	0.07
Dimethylphenanthrene	ND	0.03	0.03	ND	0.03
Phenanthrene	0.04	0.09	0.05	0.10	0.32
Anthracene	0.001	0.004	0.004	0.01	0.01
Fluoranthene	0.03	0.04	0.04	0.05	0.08
Pyrene	0.03	0.04	0.06	0.07	0.06
Benzo (a) anthracene	0.01	ND	0.02	0.02	0.04
Chrysene	ND	0.03	0.03	0.03	0.12
Indeno (1,2,3-cd) pyrene	0.02	ND	ND	0.03	ND
Benzo (b&k) fluoranthene	0.01	ND	ND	0.01	0.04
Benzo (a) pyrene	0.02	ND	ND	0.02	ND
Dibenzo (ah) anthracene	ND	ND	ND	ND	ND
Benzo (ghi) perylene	ND	ND	ND	0.08	ND
pH	6.7	7.4	7.0	7.6	7.9
% Nitrogen	0.26	0.21	0.13	0.01	0.23
% Total Organic Carbon	0.04	0.45	0.35	0.03	ND
% Moisture	1.05	1.40	0.89	1.15	0.65

Table 2 Lag phase of ^{14}C-phenanthrene mineralisation (days) in five Antarctic soils (A, B, C, D, E) at 4 ^{o}C, 12 ^{o}C, 22 ^{o}C and 22 ^{o}C slurry conditions. Errors represent standard error of mean (SEM) of triplicate samples (n = 3)

Soil-PAH contact (d)	Soil	4 ºC (days)	12 ºC (days)	22 ºC (days)	Slurry (days)
1	A	n/a	n/a	n/a	5.63 ± 0.0^{aB}
	B	n/a	n/a	n/a	10.86 ± 0.0^{bD}
	C	n/a	n/a	n/a	11.38 ± 0.0^{cC}
	D	n/a	n/a	n/a	12.47 ± 1.1^{cC}
	E	n/a	n/a	n/a	8.81 ± 0.1^{bD}
30	A	n/a	n/a	n/a	8.45 ± 0.0^{cD}
	B	n/a	n/a	n/a	7.53 ± 0.0^{cC}
	C	n/a	n/a	n/a	5.92 ± 0.1^{bB}
	D	n/a	n/a	n/a	6.94 ± 0.6^{bB}
	E	n/a	n/a	n/a	2.34 ± 0.0^{aB}
60	A	n/a	n/a	n/a	3.73 ± 0.3^{cA}
	B	n/a	n/a	n/a	4.44 ± 0.0^{dA}
	C	n/a	14.87 ± 0.6^{bA}	15.43 ± 2.1^{bA}	1.89 ± 0.1^{bA}
	D	n/a	4.76 ± 2.0^{aA}	7.28 ± 0.6^{aA}	2.28 ± 0.0^{bA}
	E	n/a	n/a	7.87 ± 1.8^{aA}	0.34 ± 0.0^{aA}
150	A	n/a	n/a	n/a	7.47 ± 0.1^{cC}
	B	n/a	n/a	n/a	6.71 ± 0.0^{bB}
	C	n/a	12.82 ± 0.7^{aA}	11.32 ± 0.3^{aA}	6.38 ± 0.3^{bB}
	D	n/a	12.91 ± 0.1^{aB}	9.39 ± 0.2^{aB}	5.22 ± 0.1^{aB}
	E	n/a	n/a	11.97 ± 2.1^{aB}	7.12 ± 0.0^{cC}

a: No statistical significant difference ($p > 0.05$) amongst soils within contact times; A: No statistical significant difference ($p > 0.05$) amongst same soils in different contact times; b, c or d: Statistical significant difference ($p < 0.05$) amongst soils within contact times; B, C or D: Statistical significant difference ($p < 0.05$) amongst same soils in different contact times.

Table 3 Maximum rates of ^{14}C-phenanthrene (% d^{-1}) mineralisation in five Antarctic soils (A, B, C, D, E) at 4 ^{o}C, 12 ^{o}C, 22 ^{o}C and 22 ^{o}C slurry conditions. Errors represent standard error of mean (SEM) of triplicate samples (n = 3)

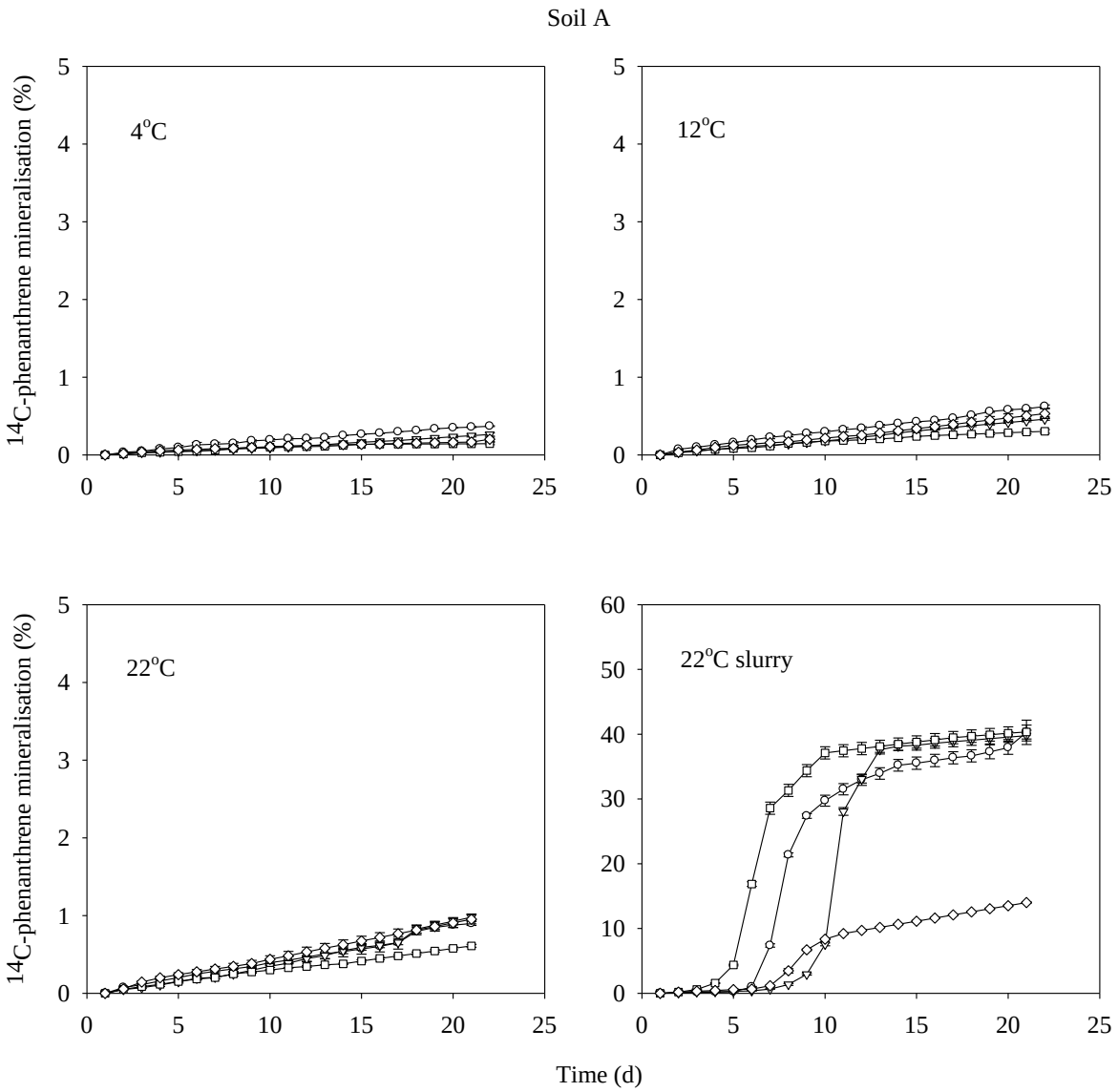
Soil-PAH contact (d)	**Soil**	**4 ^{o}C (% d^{-1})**	**12 ^{o}C (% d^{-1})**	**22 ^{o}C (% d^{-1})**	**Slurry (% d^{-1})**
1	A	0.04 ± 0.0^{aA}	0.07 ± 0.0^{aA}	0.28 ± 0.0^{aA}	14.06 ± 0.0^{bC}
	B	0.06 ± 0.0^{aA}	0.06 ± 0.0^{aA}	0.86 ± 0.0^{aB}	9.97 ± 0.1^{aC}
	C	0.03 ± 0.0^{aA}	0.06 ± 0.0^{aA}	0.16 ± 0.0^{aA}	9.91 ± 0.9^{aA}
	D	0.03 ± 0.0^{aA}	0.05 ± 0.0^{aA}	0.07 ± 0.0^{aA}	9.05 ± 0.6^{aA}
	E	0.04 ± 0.0^{aA}	0.06 ± 0.0^{aA}	0.10 ± 0.0^{aA}	24.29 ± 0.8^{cB}
30	A	0.02 ± 0.0^{aA}	0.03 ± 0.0^{aA}	0.07 ± 0.0^{aA}	20.52 ± 0.4^{dD}
	B	0.02 ± 0.0^{aA}	0.03 ± 0.0^{aA}	0.08 ± 0.0^{aA}	13.95 ± 0.4^{bD}
	C	0.02 ± 0.0^{aA}	0.03 ± 0.0^{aA}	0.10 ± 0.0^{aA}	11.74 ± 0.5^{aA}
	D	0.02 ± 0.0^{aA}	0.03 ± 0.0^{aA}	0.45 ± 0.1^{bA}	17.79 ± 0.2^{cB}
	E	0.02 ± 0.0^{aA}	0.05 ± 0.0^{aA}	0.16 ± 0.1^{aA}	27.33 ± 0.0^{eB}
60	A	0.02 ± 0.0^{aA}	0.03 ± 0.0^{aA}	0.05 ± 0.0^{aA}	12.49 ± 0.2^{cB}
	B	0.02 ± 0.0^{aA}	0.06 ± 0.0^{aA}	0.24 ± 0.2^{bA}	8.49 ± 0.2^{aB}
	C	0.02 ± 0.0^{aA}	0.74 ± 0.1^{cB}	1.47 ± 0.7^{cA}	25.69 ± 0.4^{eB}
	D	0.02 ± 0.0^{aA}	1.76 ± 0.0^{dC}	1.52 ± 0.7^{cA}	10.96 ± 0.2^{bA}
	E	0.02 ± 0.0^{aA}	0.20 ± 0.0^{bB}	3.20 ± 0.0^{dC}	19.94 ± 0.3^{dA}
150	A	0.02 ± 0.0^{aA}	0.03 ± 0.0^{aA}	0.09 ± 0.0^{aA}	3.24 ± 0.0^{aA}
	B	0.02 ± 0.0^{aA}	0.05 ± 0.0^{aA}	0.13 ± 0.0^{aB}	5.01 ± 0.3^{aA}
	C	0.03 ± 0.0^{aA}	1.33 ± 0.7^{cC}	2.32 ± 0.0^{bA}	9.50 ± 0.3^{bA}
	D	0.02 ± 0.0^{aA}	0.66 ± 0.1^{bB}	10.60 ± 0.7^{cB}	10.29 ± 0.3^{bA}
	E	0.03 ± 0.0^{aA}	2.16 ± 0.0^{dC}	2.16 ± 0.0^{bB}	21.00 ± 1.0^{cA}

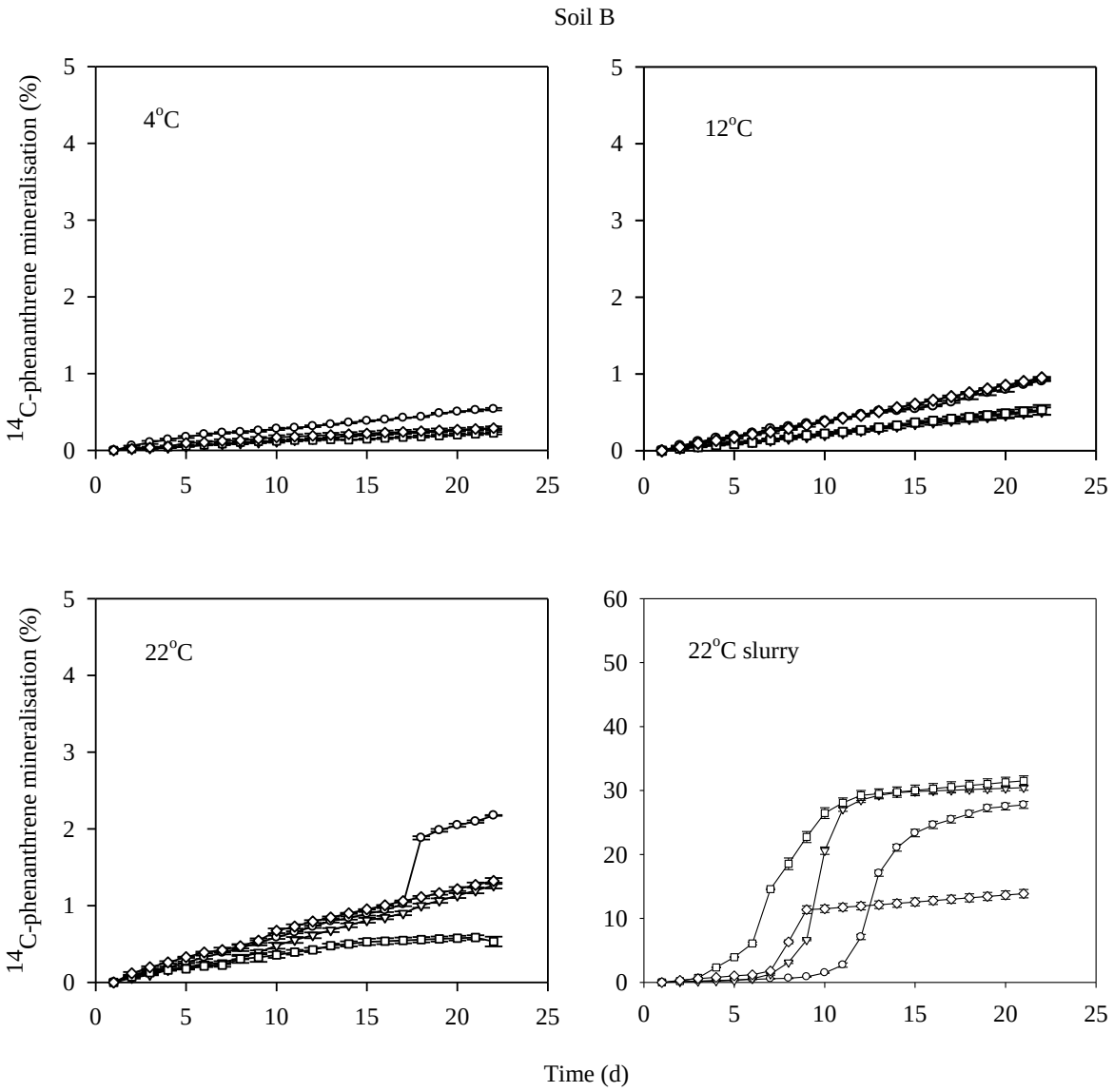
a: No statistical significant difference ($p > 0.05$) amongst soils within contact times; A: No statistical significant difference ($p > 0.05$) amongst same soils in different contact times; b, c or d: Statistical significant difference ($p < 0.05$) amongst soils within contact times; B, C or D: Statistical significant difference ($p < 0.05$) amongst same soils in different contact times.

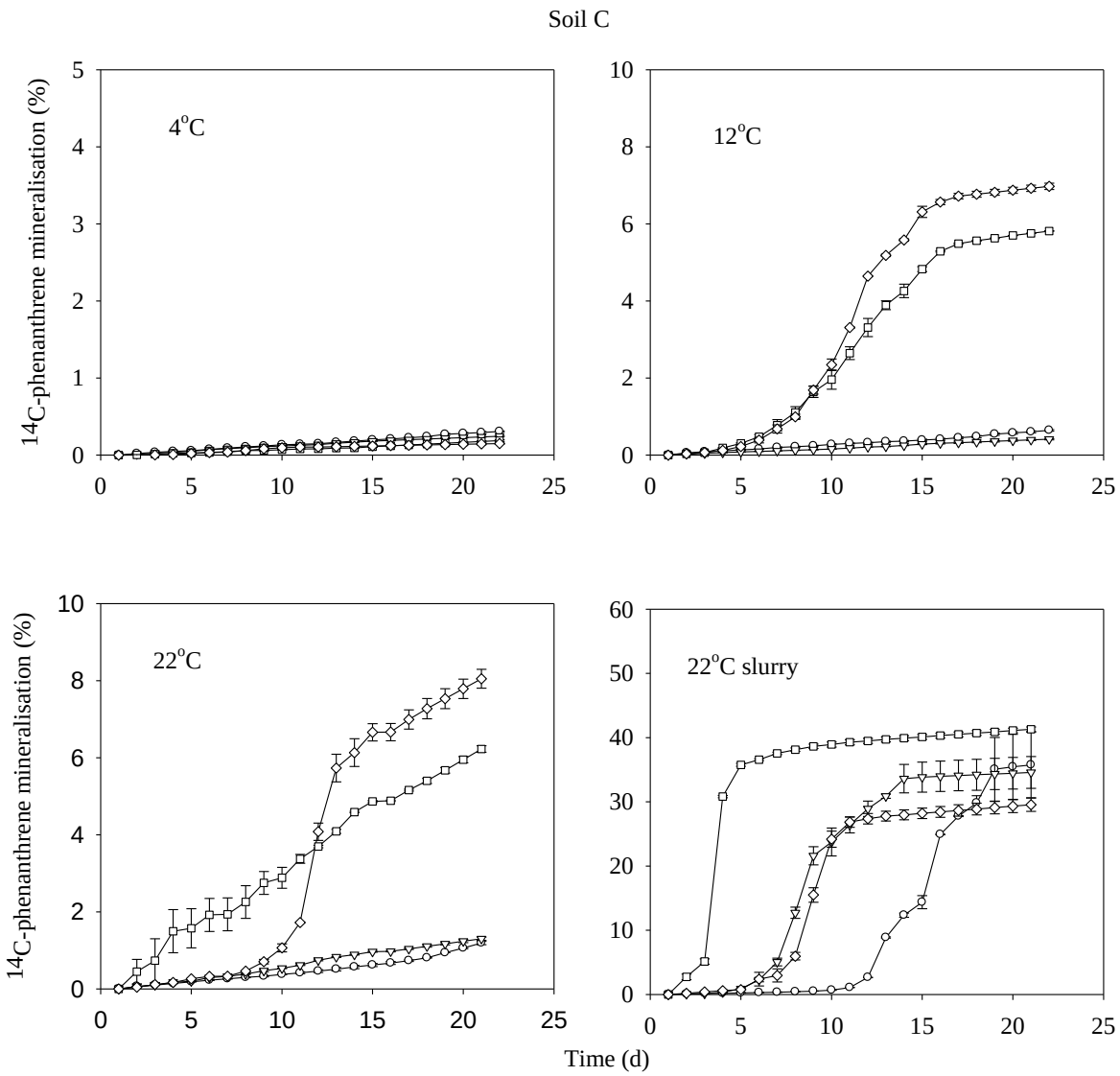
Table 4 Extents of ^{14}C-phenanthrene (%) mineralisation in five Antarctic soils (A, B, C, D, E) at 4 ^{o}C, 12 ^{o}C, 22 ^{o}C and 22 ^{o}C slurry conditions. Errors represent standard error of mean (SEM) of triplicate samples (n = 3)

Soil-PAH contact (d)	Soil	4 ^{o}C (%)	12 ^{o}C (%)	22 ^{o}C (%)	Slurry (%)
1	A	0.37 ± 0.1^{aC}	0.62 ± 0.0^{aC}	1.07 ± 0.1^{aB}	40.71 ± 1.8^{bB}
	B	0.46 ± 0.1^{aA}	0.87 ± 0.1^{bA}	2.17 ± 0.0^{cA}	28.01 ± 0.6^{aB}
	C	0.31 ± 0.0^{aA}	0.64 ± 0.0^{aA}	1.45 ± 0.2^{bA}	36.06 ± 5.2^{bA}
	D	0.36 ± 0.0^{aA}	0.59 ± 0.0^{aA}	0.94 ± 0.1^{aA}	29.77 ± 2.1^{aA}
	E	0.23 ± 0.0^{aA}	0.52 ± 0.0^{aA}	0.75 ± 0.1^{aA}	60.82 ± 1.1^{cC}
30	A	0.26 ± 0.0^{aB}	0.46 ± 0.0^{aA}	1.03 ± 0.0^{aB}	39.77 ± 0.8^{bB}
	B	0.32 ± 0.0^{aA}	0.51 ± 0.1^{aA}	1.26 ± 0.0^{aA}	30.43 ± 0.6^{aB}
	C	0.24 ± 0.0^{aA}	0.41 ± 0.0^{aA}	1.23 ± 0.2^{aA}	37.45 ± 3.9^{bA}
	D	0.21 ± 0.0^{aA}	0.30 ± 0.0^{aA}	1.35 ± 0.2^{aA}	44.12 ± 2.1^{bB}
	E	0.23 ± 0.0^{aA}	0.37 ± 0.0^{aA}	1.09 ± 0.4^{aA}	54.56 ± 2.3^{cB}
60	A	0.14 ± 0.0^{aA}	0.30 ± 0.0^{aA}	0.56 ± 0.1^{aA}	40.58 ± 1.1^{bB}
	B	0.20 ± 0.0^{aA}	0.71 ± 0.2^{aA}	2.02 ± 1.8^{aA}	31.77 ± 0.8^{aB}
	C	0.23 ± 0.0^{aA}	5.27 ± 0.7^{bB}	6.72 ± 0.6^{bB}	41.49 ± 0.4^{bA}
	D	0.14 ± 0.1^{aA}	12.21 ± 1.4^{cB}	16.83 ± 2.4^{cB}	43.32 ± 0.3^{bB}
	E	0.16 ± 0.0^{aA}	0.44 ± 0.0^{aA}	19.04 ± 0.5^{cC}	59.59 ± 1.7^{cC}
150	A	0.20 ± 0.0^{aA}	0.53 ± 0.1^{aB}	1.00 ± 0.1^{aB}	14.48 ± 0.1^{aA}
	B	0.29 ± 0.0^{aA}	0.95 ± 0.0^{aA}	1.32 ± 0.0^{aA}	14.08 ± 0.7^{aA}
	C	0.15 ± 0.0^{aA}	6.97 ± 0.1^{bC}	8.00 ± 0.3^{bB}	29.77 ± 1.1^{bA}
	D	0.27 ± 0.0^{aA}	8.57 ± 1.1^{bB}	24.82 ± 1.2^{cB}	38.82 ± 1.3^{bB}
	E	0.10 ± 0.0^{aA}	3.05 ± 0.2^{aB}	8.27 ± 0.3^{bB}	33.49 ± 0.6^{bA}

a: No statistical significant difference ($p > 0.05$) amongst soils within contact times; A: No statistical significant difference ($p > 0.05$) amongst same soils in different contact times; b, c or d: Statistical significant difference ($p < 0.05$) amongst soils within contact times; B, C or D: Statistical significant difference ($p < 0.05$) amongst same soils in different contact times.


Soil A
4°C
12°C
22°C
22°C slurry
14C-phenanthrene mineralisation (%)
Time (d)


Soil B
4°C
12°C
22°C
22°C slurry
14C-phenanthrene mineralisation (%)
Time (d)


Soil C
4°C
12°C
22°C
22°C slurry
14C-phenanthrene mineralisation (%)
Time (d)

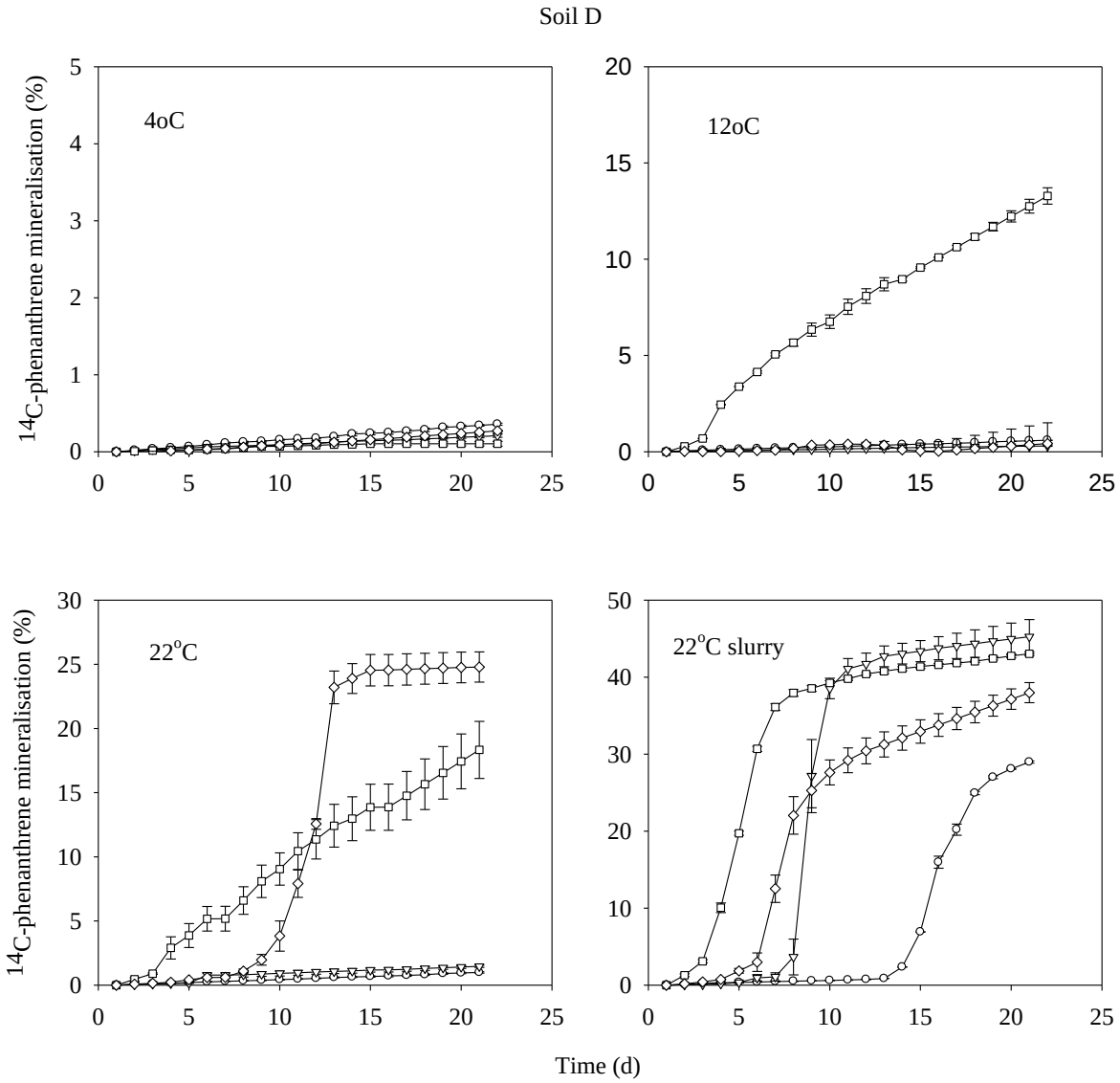


Figure 4 Mineralisation of ^{14}C-phenanthrene in soil D under different temperature regimes (4ºC, 12ºC, 22ºC, 22ºC slurry) after 1 (○), 30 (▽), 60 (□) and 150 (◊) days contact times. Error bars represent standard error of mineralisation (SEM) (n = 3).

Soil E

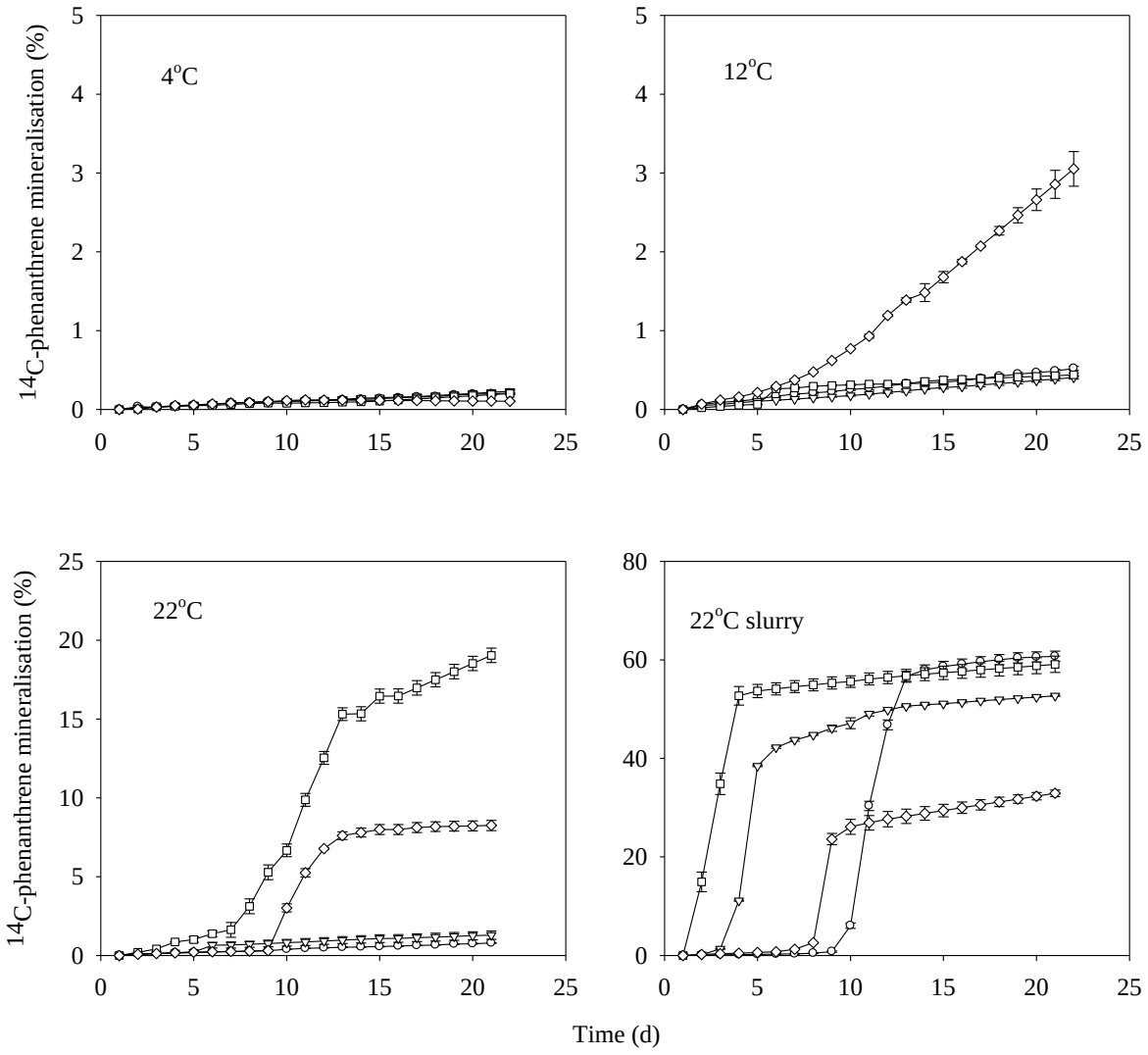

4°C
12°C
22°C
22°C slurry
14C-phenanthrene mineralisation (%)
Time (d)

Figure legends

Figure 1 Mineralisation of ^{14}C-phenanthrene in soil A under different temperature regimes (4ºC, 12ºC, 22ºC, 22ºC slurry) after 1 (○), 30 (▽), 60 (□) and 150 (◊) days contact times. Error bars represent standard error of mineralisation (SEM) (n = 3).

Figure 2 Mineralisation of ^{14}C-phenanthrene in soil B under different temperature regimes (4ºC, 12ºC, 22ºC, 22ºC slurry) after 1 (○), 30 (▽), 60 (□) and 150 (◊) days contact times. Error bars represent standard error of mineralisation (SEM) (n = 3).

Figure 3 Mineralisation of ^{14}C-phenanthrene in soil C under different temperature regimes (4ºC, 12ºC, 22ºC, 22ºC slurry) after 1 (○), 30 (▽), 60 (□) and 150 (◊) days contact times. Error bars represent standard error of mineralisation (SEM) (n = 3).

Figure 4 Mineralisation of ^{14}C-phenanthrene in soil D under different temperature regimes (4ºC, 12ºC, 22ºC, 22ºC slurry) after 1 (○), 30 (▽), 60 (□) and 150 (◊) days contact times. Error bars represent standard error of mineralisation (SEM) (n = 3).

Figure 5 Mineralisation of ^{14}C-phenanthrene in soil E under different temperature regimes (4ºC, 12ºC, 22ºC, 22ºC slurry) after 1 (○), 30 ( ), 60 (□) and 150 (◊) days contact times. Error bars represent standard error of mineralisation (SEM) (n = 3).