View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Lancaster E-Prints

1
Effects of pre-exposure on the indigenous biodegradation of ^{14}C-phenanthrene in
2
Antarctic soils

3 Uchechukwu V. Okere[1], Ana Cabrerizo[2], Jordi Dachs[2], Uchenna O. Ogbonnaya[1,3], Kevin C.
4 Jones[1], Kirk T. Semple[1]*

5

6 [1] Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK.

7 [2] Department of Environmental Chemistry, IDAEA-CSIC Jordi Girona 18-26, Barcelona
8 08034, Catalonia, Spain

9 [3] Department of Soil Sciences and Land Resource Management, Federal University Oye-
10 Ekiti, Nigeria

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28 *Corresponding author's mail address: k.semple@lancaster.ac.uk

29 **Abstract**

30 The aim of this study was to investigate the biodegradation of phenanthrene in five Antarctic
31 soils over 150 days at various temperatures and under slurry conditions. The development of
32 catabolic activity was measured over time (1, 30, 60, 150 days) by the addition of ^{14}C-
33 phenanthrene and measuring changes in the lag phases, rates and extents of ^{14}C-phenanthrene
34 degradation. As the temperature increased (4 ºC, 12 ºC, 22 ºC, 22 ºC slurry), the highest
35 extents of ^{14}C-phenanthrene mineralisation increased significantly (0.46%, 12.21%, 24.82%,
36 60.81%), respectively. This was due to changes in the water availability and ^{14}C-
37 phenanthrene dissolution in aqueous phase, thus enhancing bioaccessibility of the
38 contaminant to indigenous microorganisms within the soil. High catabolic activities can
39 develop in Antarctic soils where appropriate conditions are ensured. However, further studies
40 are however needed to explore the changes in microbial community structure that occur at
41 different incubation temperatures.

42

43

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

45

46

47 1. **Introduction**

48 Polycyclic aromatic hydrocarbons (PAHs) are an important class of environmental pollutants
49 (Doick et al., 2003). Their potential for long range atmospheric transport (Prevedouros et al.,
50 2004), ubiquitous presence in the atmosphere (Garrido et al., 2014), marine (Latimer and
51 Zheng, 2003) and soil environments (Wilcke, 2007), low aqueous solubility, high octanol
52 water coefficient (log K_{ow}) and adverse health effects (Kim et al., 2013) has fuelled research
53 interest into their sources and fate in varying environments. The fate of PAHs in the soil
54 environment is critical to the amount of PAHs present in the total environment. This is
55 because about 90% of the global environmental PAHs are stored in soils (Wild and Jones,
56 1995; Agarwal et al., 2009). Despite this, soils can serve as a disturbing source of PAHs to
57 the atmosphere (Cousins and Jones, 1998; Wang et al., 2010). As a result, PAH
58 concentrations in soils have been found to correspond to concentrations in the atmospheric
59 environment (Zhao et al., 2015).

60 PAHs are removed from soil primarily through microbial activity as these aromatics represent
61 sources of carbon and energy for microbial metabolism (Semple et al., 2006; Couling et al.,
62 2010; Guo et al., 2010). As a result, PAH-degrading microorganisms have been isolated from
63 many different soils, including tropical (Obayori et al., 2008; Obayori et al., 2009; Guo et al.,
64 2010; Isaac et al., 2013), temperate (Johnsen et al., 2006; Ogbonnaya et al., 2014a) and
65 extreme temperature environments, such as cold (Baraniecki et al., 2002) and hot deserts
66 (Abed et al., 2015a; 2015b). In order for microbial degradation of PAHs to occur, the
67 presence of microorganisms with the appropriate genetic potential is essential (Peng et al.,
68 2008). The microorganisms must be in the same environment as the PAH and the PAH must
69 be able to be physically transferred to the site of metabolism in the microorganism (Macleod
70 et al., 2001). Bioavailability is also important and depends on the physicochemical properties
71 and concentration of the PAHs (Guo et al., 2010; Sayara et al., 2010), the properties of the

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

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

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

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

100

101 ## 2. Materials and methods

102 ### 2.1 *Materials*

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

110

111 ### 2.2 *Soils sampling and bulk characterization*

112 Composite topsoil (0-5 cm) samples (5) were collected using a stainless-steel corer from
113 different locations of Livingstone Island, Antarctica and labelled A – E. According to sample
114 transportation standards, the samples were frozen (-20 ºC) in sterile glass jars and then
115 transported to Lancaster Environment Centre. The soils were allowed to defrost and further
116 air-dried and passed through a 2 mm sieve to remove stones and fibrous material. Then the
117 soils were subject to physical and chemical analysis to determine their properties (Table 1).
118 Soil redox, soil pH and soil moisture content were measured by standard methods according
119 to Cabrerizo et al. (2011). Particle size analysis and calculations were determined according
120 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,

169 30, 60 and 150 days contact times. Pre-exposed soils (10 g) rehydrated to 40-60% water
170 holding capacity were placed in a respirometer and spiked with ^{12}C- (>99.6%) and ^{14}C-
171 phenanthrene (80 Bq ^{14}C-phenanthrene g^{-1} soil) using toluene as a carrier solvent. A 7 ml
172 scintillation vial containing 1 M NaOH was attached to the screw cap to serve as a CO_2 trap.
173 The respirometers were stored in the dark at the respective temperatures which the soils were
174 exposed (4 ºC, 12 ºC, 22 ºC). A slurry system was also set up containing 30 ml mineral basal
175 salts (MBS) medium as Ogbonnaya et al. (2014b) and placed on a SANYO® Gallenkamp
176 orbital incubator set at 100 rpm and 22 °C to agitate and ensure adequate mixing over the
177 period of the incubation. NaOH traps were replaced every 24 h, after which 6 ml of Ultima
178 Gold scintillation cocktail was added to each spent trap and the contents analysed on a
179 Packard Canberra Tri-Carb 2250CA liquid scintillation counter. The incubation lasted for 21
180 days. Lag phases were measured as the time (days) before ^{14}C-phenanthrene mineralisation
181 reached 5%. Analytical blanks containing no ^{14}C-phenanthrene were used for the
182 determination of levels of background radioactivity.

183

184 2.7 *Statistical analysis*

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

189

190 3.0 **Results**

191 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 ($\geq$ 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 ($\leq$ 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

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

218 At a higher temperature (12 ºC), the lag phase was not observed until after 60 days contact
219 time in soils C (14.87 days) and D (4.76 days), where the lag phase of soil D was statistically
220 the shortest ($P < 0.001$) compared to other soils under 12 ºC assay condition (Table 2).
221 However, as the contact time increased to 150 days, the lag phase in soil D statistically
222 increased ($P < 0.001$) following further exposure to 12.91 days which was statistically similar
223 to other soil C (Table 2). Similar to 4 ºC soil assay, the maximum rate of ^{14}C-phenanthrene in
224 12 ºC soil did not exceed 0.07% d^{-1} following 1 and 30 days contact times, but after pre-
225 exposure at 60 days, maximum rate of mineralisation significantly increased to 0.74%, 1.76%
226 and 0.20% d^{-1} in soils C, D and E, respectively (Table 3). Further increase in contact time did
227 not sustain the rates of mineralisation, rather led to decreased rates, except for soil C (Table
228 3). The extents of ^{14}C-Phenanthrene mineralisation after 1 d contact time were similar in all
229 five soils (Figures 1-5, Table 2). After 30 days contact time, the extents of ^{14}C-phenanthrene
230 mineralisation in all the soils decreased, but increasing contact time to 60 days resulted in
231 significant increases ($P < 0.05$) in ^{14}C-phenanthrene mineralisation in soils C (5.3%) and D
232 (12.2%) alone. This was maintained after the 150 days pre-exposure in soils C and D (Table
233 4).

234 At 22 ºC, lag phases were observed and they increased in soils D and E as contact time
235 increased but there was statistical increase ($P < 0.05$) only at 150 days contact time compared
236 to other time points in both soils. Lag phase insignificantly decreased ($P = 0.49$) in soil C as
237 contact time increased from 60 to 150 days (Table 2). Maximum rates of ^{14}C-phenanthrene
238 mineralisation increased ($P < 0.05$) with contact time in soils C, D and E compared to the
239 shorter contact times (1 and 30 days) (Table 3). Microbial catabolic activity in soil D
240 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

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

268

269 ## 4.0 **Discussion**

270 ### 4.1 *Soil physico-chemical properties*

271 As in previous studies, soils collected from Livingstone Island of Antarctica Island, distant
272 from persistent human activities were characterised by extremely low nutrient, organic
273 carbon and moisture conditions (Campbell and Claridge, 1987; Okere et al., 2012b). Losses
274 of these components are common with coarse sandy soils, clays are minor in such soils due to
275 the dominance of physical weathering processes over chemical weathering (Egli et al., 2008;
276 Spinola et al., 2017). Apparently, vegetative cover and biological presence were found to be
277 limited within the studied region, which further contributed to the low level organic carbon
278 content, but does not rule out microbial presence (Okere et al., 2012b). The soil organisms
279 encounter extremely low water and nutrient content, very low temperatures with ice
280 formations, freeze–thaw cycles, prolonged darkness in winter and short summer spells (Cary
281 et al., 2010). Despite the harsh environmental conditions, the alkaline pH condition is
282 favourable for bacterial growth and activities (Aislabie et al., 2001; Baraniecki et al., 2002;
283 Okere et al., 2012b). PAHs were found in all the five soils at levels (very low) similar to
284 those reported in uncontaminated/pristine soils (Johnsen and Karlson, 2005; Cabrerizo et al.,
285 2012; Okere et al., 2012b).

286

287 ### 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-

337 phenanthrene mineralisation as the temperature increased to 22 ºC, due to increased water
338 availability and phenanthrene dissolution in aqueous phase, thus enhancing bioaccessibility
339 of the contaminants to indigenous microorganisms (ten Hulscher and Cornelissen, 1996;
340 Coulon et al., 2005). In respect to catabolic activities, lag phases were observed in soils C and
341 D at 12 ºC after 60 to 150 days contact times and under the 22 ºC (soils C, D, E) incubation
342 conditions. This accurately coincided with extents of ^{14}C-phenanthrene mineralisation in the
343 said soils, where rates of mineralisation exceeded 1% d^{-1}, extents of mineralisation exceeded
344 5%. Despite all the soils in this study being collected under the same climatic conditions
345 (Antarctica) and exhibited similar physico-chemical properties (N, TOC, pH, texture,
346 moisture content) which were invariably low, catabolic activities varied with contact time and
347 temperature. Although not investigated, this suggests that the different soils contained
348 different spectra and density of psychrophilic and psychrotrophic microorganisms capable of
349 degrading phenanthrene (Eriksson et al., 2003; Antizar-Ladislao et al., 2008).
350
351 Catabolic activities were mainly pronounced under slurry conditions at 22 ºC. The lag phases
352 in each soil showed a decreasing trend as the incubation time increased until 60 days contact
353 time indicating an adaptation of the indigenous microorganisms to the presence of
354 phenanthrene (Couling et al., 2010; Ogbonnaya et al., 2014b; Oyelami et al., 2015).
355 Microbial adaptation would have been through increase in microbial population (growth of
356 mesophiles), catabolic enzyme induction and transgenic manipulations degrading populations
357 (Top and Springael, 2003; Ogbonnaya 2014b). Microbial adaptations followed a sigmoidal
358 pattern and the period took much longer time (60 days) to be below 5 days compared to
359 adaptation period of phenanthrene mineralisation in UK soils (Couling et al., 2010; Rhodes et
360 al., 2010; Ogbonnaya et al., 2014b) despite having lower organic carbon content and being
361 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

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

389

390

391 ## 5.0 **Conclusion**

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

400

401

402 ## **References**

403 Abed, R.M.M., Al-Kindi, S., Al-Kharusi, S., 2015a. Diversity of bacterial communities along
404 a petroleum contamination gradient in desert soils. Microbial Ecology 69, 95-105.
405 Abed, R.M.M., Al-Kharusi, S, Al-Hinai, M., 2015b. Effect of biostimulation, temperature
406 and salinity on respiration activities and bacterial community composition in an oil polluted
407 desert soil. International Biodeterioration and Biodegradation 98, 43-52.
408 Agarwal, T., Khillare, P.S., Shridhar, V., Ray, S., 2009. Pattern, sources and toxic potential of PAHs
409 in the agricultural soils of Delhi, India. Journal of Hazardous Materials 163, 1033-1039.
410 Aislabie, J., Balks, M., Astori, N., Stevenson, G., Symons, R., 1999. Polycyclic aromatic
411 hydrocarbons in fuel-oil contaminated soils, Antarctica. Chemosphere 39, 2201-2207.
412 Aislabie, J., Fraser, R., Duncan, S., Farrell, R.L., 2001. Effects of oil spills on microbial heterotrophs
413 in Antarctic soils. Polar Biology 24, 308-313.
414 Anderson, C., Rozzi, R., Torres-Mura, J., McGehee, S., Sherriffs, M., Schüttler, E., Rosemond, A.,
415 2006. Exotic Vertebrate Fauna in the Remote and Pristine Sub-Antarctic Cape Horn Archipelago,
416 Chile. Biodiversity and Conservation 15, 3295-3313.

417 Antizar-Ladislao, B., Spanova, K., Beck, A.J., Russell, N.J., 2008. Microbial community structure
418 changes during bioremediation of PAHs in an aged coal-tar contaminated soil by in-vessel
419 composting. International Biodeterioration and Biodegradation 61, 357-364.
420 Baraniecki, C.A., Aislabie, J., Foght, J.M., 2002. Characterization of *Sphingomonas sp. Ant 17*, an
421 Aromatic Hydrocarbon-Degrading Bacterium Isolated from Antarctic Soil. Microbial Ecology 43, 44-
422 54.
423 Bockheim, J.G., 1997. Properties and Classification of Cold Desert Soils from Antarctica. Soil
424 Science Society of America Journal 61, 224-231.
425 Bosma, T.N.P., Middeldorp, P.J.M., Schraa, G., Zehnder, A.J.B., 1996. Mass Transfer Limitation of
426 Biotransformation: Quantifying Bioavailability. Environmental Science and Technology 31, 248-252.
427 Bossert, I., Bartha, R., 1984. The Fat of Petroleum in Soil Ecosystems. In: Atlas, R.M., Ed.,
428 Petroleum Microbiology, Macmillan, New York, 440-445.
429 Cabrerizo, A., Dachs, J., Barcel, D., 2009. Development of a Soil Fugacity Sampler for Determination
430 of Air−Soil Partitioning of Persistent Organic Pollutants under Field Controlled Conditions.
431 Environmental Science and Technology 43, 8257-8263.
432 Cabrerizo, A., Dachs, J., Moeckel, C., Ojeda, M.a.-J., Caballero, G., Barceló, D., Jones, K.C., 2011.
433 Ubiquitous Net Volatilization of Polycyclic Aromatic Hydrocarbons from Soils and Parameters
434 Influencing Their Soil−Air Partitioning. Environmentl Science and Technology 45, 4740-4747.
435 Cabrerizo, A., Dachs, J., Barcelò, D., Jones, K.C., 2012. Influence of organic matter content and
436 human activities on the occurrence of organic pollutants in Antarctic soils, lichens, grass, and mosses.
437 Environmental Science and Technology 46, 1396-1405.
438 Campbell, I.B., Claridge, G.G.C., 1987. Antarctica: soils, weathering processes and environment.
439 Elsevier, Amsterdam, 406 pp.
440 Campbell, I.B., Claridge, G.G.C., 2009. Antarctic permafrost soils, in: Margesin, R. (Ed.), Permafrost
441 soils. Springer-Verlag Berlin and Heidelberg GmbH & Co. K, pp. 17-31.
442 Cary, S.C., McDonald, I.R., Barrett, J.E., Cowan, D.A., 2010. On the rocks: the microbiology of
443 Antarctic Dry Valley soils. Nature Reviews Microbiology 8, 129–138
444 Couling, N.R., Towell, M.G., Semple, K.T., 2010. Biodegradation of PAHs in soil: Influence of
445 chemical structure, concentration and multiple amendment. Environmental Pollution 158, 3411-3420.
446 Coulon, F., Pelletier, E., Gourhant, L., Delille, D., 2005. Effects of nutrient and temperature on
447 degradation of petroleum hydrocarbons in contaminated sub-Antarctic soil. Chemosphere 58, 1439-
448 1448.
449 Cousins, I.T., Jones, K.C., 1998. Air-soil exchange of semi-volatile organic compounds (SOCs) in the
450 UK. Environmental Pollution 102, 105-118.
451 Cowan, D.A., Makhalanyane, T.P., Dennis, P.G., Hopkins, D.W. 2014. Microbial ecology and
452 biogeochemistry of continental Antarctic soils. Frontiers in Microbiology 5:154.
453 D'Amico, S., Collins, T., Marx, J., Feller, G., Gerday, C., 2006. Psychrophillic microorganisms:
454 Challenges for life. EMBO Reports 7, 385-389.
455 Doick, K.J. Semple, K.T., 2003. The effect of soil: water ratios on the mineralisation of phenanthrene:
456 LNAPL mixtures in soil. FEMS Microbiology Letters 220, 29-33.
457 Doick, K.J., Lee, P.H., Semple, K.T., 2003. Assessment of spiking procedures for the introduction of
458 a phenanthrene-LNAPL mixture into field-wet soil. Environmental Pollution 126, 399-406.
459 Egli, M., Mirabella, A., Sartori, G., 2008. The role of climate and vegetation in weathering and clay
460 mineral formation in late Quaternary soils of the Swiss and Italian Alps. Geomorphology 102, 307-
461 324.
462 Eriksson, M., Sodersten, E., Yu, Z., Dalhammar, G., Mohn, W.W., 2003. Degradation of polycyclic
463 aromatic hydrocarbons at low temperature under aerobic and nitrate-reducing conditions in
464 enrichment cultures from Northern soils.Applied and Envrionmental Microbiology 69, 275–284.
465 Garrido, A., Jimenez-Guerrero, P., Ratola, N., 2014. Levels, trends and health concerns of
466 atmospheric PAHs in Europe. Atmospheric Environment 99, 474-484.
467 Gee, G.W., Bauder, J.W., 1979. Particle Size Analysis by Hydrometer: A Simplified Method for
468 Routine Textural Analysis and a Sensitivity Test of Measurement Parameters. Soil Science Society of
469 America Journal 43, 1004-1007.
470 Gee, G.W., Bauder, J.W., 1986. Particle-size analysis, in: Klute, A. (Ed.), Methods of soil analysis.
471 Part 1. Physical and Mineralogical Methods. ASA and SSSA, Madison, WI, pp. 383–411.

472 Guo, C., Dang, Z., Wong, Y., Tam, N.F., 2010. Biodegradation ability and dioxgenase genes of PAH-
473 degrading Sphingomonas and Mycobacterium strains isolated from mangrove sediments. International
474 Biodeterioration and Biodegradation 64, 419-426.
475 ten Hulscher, T.E.M., Cornelissen, G., 1996. Effect of temperature on sorption equilibrium and
476 sorption kinetics of organic micropollutants - a review. Chemosphere 32, 609-626.
477 Isaac, P., Sánchez, L.A., Bourguignon, N., Cabral, M.E., Ferrero, M.A., 2013. Indigenous PAH-
478 degrading bacteria from oil-polluted sediments in Caleta Cordova, Patagonia Argentina. International
479 Biodeterioration and Biodegradation 82, 207-214.
480 Johnsen, A.R., Karlson, U., 2005. PAH degradation capacity of soil microbial communities: does it
481 depend on PAH exposure? Microbial Ecology 50, 488-495.
482 Johnsen, A.R., de Lipthay, J.R., Reichenberg, F., Sorensen, S.J., Andersen, O., Christensen, P.,
483 Binderup, M.L., Jacobsen, C.S., 2006. Biodegradation, bioaccessibility, and genotoxicity of diffuse
484 polycyclic aromatic hydrocarbon (PAH) pollution at a motorway site. Environmental Science and
485 Technology 40, 3293-3298.
486 Kim, K.H., Jahan, S.A., Kabir, E., Brown, R.J.C., 2013. A review of airborne polycyclic aromatic
487 hydrocarbons (PAHs) and their human health effects. Environment International 60, 71-80.
488 Latimer, J.S., Zheng, J., 2003. The Sources, Transport, and Fate of PAHs in the Marine Environment,
489 PAHs: An Ecotoxicological Perspective. John Wiley & Sons, Ltd, pp. 7-33.
490 Leahy, G.J., Colwell, R.R., 1990. Microbial degradation of hydrocarbons in the environment.
491 Microbiological Reviews 54, 305-315.
492 Leonardi, V., Šašek, V., Petruccioli, M., D'Annibale, A., Erbanová, P., Cajthaml, T., 2007
493 Bioavailability modification and fungal biodegradation of PAHs in aged industrial soils. International
494 Biodeterioration and Biodegradation 60, 165-170.
495 MacLeod, C.J.A., Morriss, A.W.J., Semple, K.T., 2001. The role of microorganisms in ecological risk
496 assessment of hydrophobic organic contaminants in soils, Advances in Applied Microbiology.
497 Academic Press, pp. 171-212.
498 Margesin, R., Schinner, F., 2001. Biodegradation and bioremediation of hydrocarbons in extreme
499 environments. Applied Microbiology and Biotechnology 56, 650-663.
500 Obayori, O., Ilori, M., Adebusoye, S., Oyetibo, G., Amund, O., 2008. Pyrene-degradation potentials
501 of *Pseudomonas* species isolated from polluted tropical soils. World Journal of Microbiology and
502 Biotechnology 24, 2639-2646.
503 Obayori, O.S., Adebusoye, S.A., Adewale, A.O., Oyetibo, G.O., Oluyemi, O.O., Amokun, R.A., Ilori,
504 M.O., 2009. Differential degradation of crude oil (Bonny Light) by four *Pseudomonas* strains. Journal
505 of Environmental Sciences 21, 243-248.
506 Ogbonnaya, O.U., Adebisi, O.O., Semple, K.T., 2014a. The impact of biochar on the bioaccessibility
507 of ^{14}C-phenanthrene in aged soil. Environmental Science: Processes & Impacts 16, 2635-2643.
508 Ogbonnaya, U., Oyelami, A., Matthews, J., Adebisi, O., Semple, K.T., 2014b. Influence of Wood
509 Biochar on Phenanthrene Catabolism in Soils. Environments 1, 60-74.
510 Ogbonnaya, U., Thomas, J., Fasina, S.A., Semple, K.T., 2016. Impact of two contrasting biochars on
511 the bioaccessibility of ^{14}C-naphthalene in soil. Environmental Technology and Innovation, 6, 80-93.
512 Okere, U.V., Cabrerizo, A., Dachs, J., Jones, K.C., Semple, K.T., 2012a. Biodegradation of
513 phenanthrene by indigenous microorganisms in soils from Livingstone Island, Antarctica. FEMS
514 Microbiology Letters 329, 69-77.
515 Okere, U.V., Semple, K.T., 2012b. Biodegradation of PAHs in 'Pristine' Soils from Different
516 Climatic Regions. Journal of Bioremediation and Biodegradation S1:006.
517 Oyelami, A.O., Ogbonnaya, U., Muotoh, C., Semple, K.T., 2015. Impact of activated carbon on the
518 catabolism of ^{14}C-phenanthrene in soil. Environmental Science: Processes & Impacts 17, 1173-1181.
519 Peng, R.-H., Xiong, A.-S., Xue, Y., Fu, X.-Y., Gao, F., Zhao, W., Tian, Y.-S., Yao, Q.-H., 2008.
520 Microbial biodegradation of polyaromatic hydrocarbons. FEMS Microbiology Reviews 32, 927-955.
521 Pointing, S.B., Chan, Y., Lacap, D.C., Lau, M.C., Jurgens, J.A., Farrell, R.L., 2009. Highly
522 specialized microbial diversity in hyper-arid polar desert. Proceedings of National Academy of
523 Science United States of America. 106, 19964-19969.
524 Prevedouros, K., Brorström-Lundén, E., J. Halsall, C., Jones, K.C., Lee, R.G.M., Sweetman, A.J.,
525 2004. Seasonal and long-term trends in atmospheric PAH concentrations: evidence and implications.
526 Environmental Pollution 128, 17-27.

527 Qu, X., Liu, P., Zhu, D., 2008. Enhanced sorption of polycyclic aromatic hydrocarbons to tetra-alkyl
528 ammonium modified smectites via cation−π interactions. Environmental Science and Technology 42,
529 1109–1116.
530 Rao, S., Chan, Y., Lacap, D.C., Hyde, K.D., Pointing, S.B., Farrell, R.L., 2012. Low-diversity fungal
531 assemblage in an Antarctic dry valleys soil. Polar Biology 35, 567-574.
532 Reid, B.J., MacLeod, C.J.A., Lee, P.H., Morriss, A.W.J., Stokes, J.D., Semple, K.T., 2001. A simple
533 ^{14}C-respirometric method for assessing microbial catabolic potential and contaminant bioavailability.
534 FEMS Microbiology Letters 196, 141-146.
535 Rhodes, A.H., Owen, S.M., Semple, K.T., 2007. Biodegradation of 2,4-dichlorophenol in the presence
536 of volatile organic compounds in soils under different vegetation types. FEMS Microbiology Letters
537 269, 323-330.
538 Rhodes, A., McAllister, L.E., Semple, K.T., 2010. Linking desorption kinetics to phenanthrene
539 biodegradation in soil. Environmental Pollution 158, 1348-1353.
540 Sayara, T., Pognani, M., Sarrà, M., Sánchez, A., 2010. Anaerobic degradation of PAHs in soil:
541 Impacts of concentration and amendment stability on the PAHs degradation and biogas production.
542 International Biodeterioration & Biodegradation 64, 286-292.
543 Sayara, T., Borràs, E., Caminal, G., Sarrà, M., Sánchez, A., 2011. Bioremediation of PAHs-
544 contaminated soil through composting: Influence of bioaugmentation and biostimulation on
545 contaminant biodegradation. International Biodeterioration and Biodegradation 65, 859-865.
546 Semple, K.T., Morriss, A.W.J., Paton, G.I., 2003. Bioavailability of hydrophobic organic
547 contaminants in soils: fundamental concepts and techniques for analysis. European Journal of Soil
548 Science 54, 809-818.
549 Semple, K.T., Dew, N.M., Doick, K.J., Rhodes, A., 2006. Can microbial mineralization be used to
550 estimate microbial availability of organic contaminants in soil? Environmental Pollution 140, 164-
551 172.
552 Semple, K.T., Doick, K.J., Wick, L.Y., Harms, H., 2007. Microbial interactions with organic
553 contaminants in soil: definitions, processes and measurement. Environmental Pollution 150, 166-176.
554 Spinola, D.N., Portes, R.D.C., Schaefer, C.E.G.R., Solleiro-Rebolledo, E., Pi-Puig, T., Kühn, P.,
555 2017. Eocene paleosols on King George Island, Maritime Antarctica: Macromorphology,
556 micromorphology and mineralogy. Catena 152, 69-81.
557 Top, E.M., Springael, D., 2003. The role of mobile genetic elements in bacterial adaptation to
558 xenobiotic organic compounds. Current Opinion in Biotechnology 14, 262-269.
559 van der Meer, J.R., de Vos, W.M., Harayama, S., Zehnder, A.J., 1992. Molecular mechanisms of
560 genetic adaptation to xenobiotic compounds. Microbiological Reviews 56, 677-694.
561 Wang, K., Xing, B., 2005. Structural and sorption characteristics of adsorbed humic acid on clay
562 minerals. Journal of Environmental Quality 34, 342-349.
563 Wang, X., Sato, T., Xing, B., 2005. Sorption and displacement of pyrene in soils and sediments.
564 Environmental Science and Technology 39, 8712-8718.
565 Wang, W., Staci L. Massey Simonich, S.L.M., Xue, M., Zhao, J., Zhang, N., Wang, R., Cao, J., Tao,
566 S., 2010. Concentrations, sources and spatial distribution of polycyclic aromatic hydrocarbons in soils
567 from Beijing, Tianjin and surrounding areas, North China. Environmental Pollution 158, 1245-1251.
568 Wilcke, W., 2000. Polycyclic aromatic hydrocarbons (PAHs) in soil — a review. Journal of Plant
569 Nutrition and Soil Science 163, 229-248.
570 Wilcke, W., 2007. Global patterns of polycyclic aromatic hydrocarbons (PAHs) in soil. Geoderma
571 141, 157-166.
572 Wild, S.R., Jones, K.C., 1995. Polynuclear aromatic hydrocarbons in the United Kingdom
573 environment: A preliminary source inventory and budget. Environmental Pollution 88, 91-108.
574 Zhang, W., Zhuang, L., Yuan, Y., Tong, L., Tsang, D.C.W., 2011. Enhancement of phenanthrene
575 adsorption on a clayey soil and clay minerals by coexisting lead or cadmium. Chemosphere 83, 302-
576 310.
577 Zhao, X., Kim, S.K., Zhu, W., Kannan, N., Li, D., 2015. Long-range atmospheric transport and the
578 distribution of polycyclic aromatic hydrocarbons in Changbai Mountain. Chemosphere 119, 289-294
579 Zwietering, M.H., Jongenburger, I., Rombouts, F.M., van 't Riet, K., 1990. Modeling of the Bacterial
580 Growth Curve. Applied and Environmental Microbiology 56, 1875-1881.

581
582
583
584
585
586
587
588

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 ºC, 12 ºC, 22 ºC and 22 ºC 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 ºC, 12 ºC, 22 ºC and 22 ºC slurry conditions. Errors represent standard error of mean (SEM) of triplicate samples (n = 3)

Soil-PAH contact (d)	**Soil**	**4 ºC (% d^{-1})**	**12 ºC (% d^{-1})**	**22 ºC (% 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}

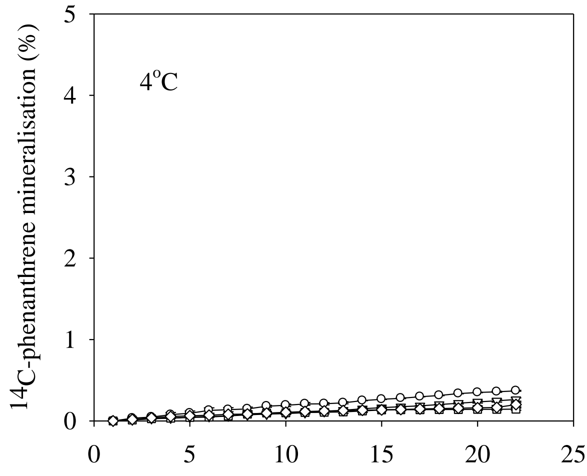
a: No statistical significant difference ($p > 0.05$) amongst soils within contact times; A: No statistical significant difference ($p > 0.05$) amongst same soils in different contact times; b, c or d: Statistical significant difference ($p < 0.05$) amongst soils within contact times; B, C or D: Statistical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

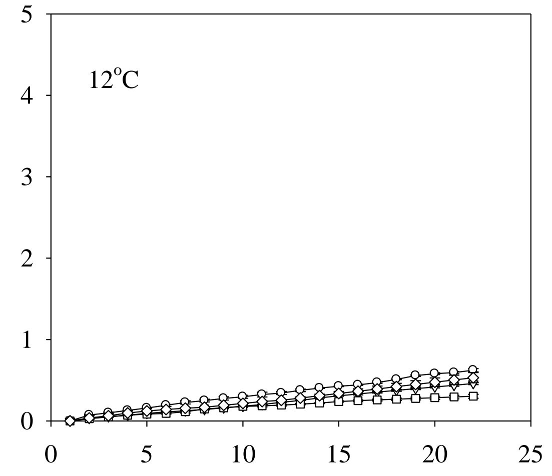
Table 4 Extents of ^{14}C-phenanthrene (%) mineralisation in five Antarctic soils (A, B, C, D, E) at 4 ºC, 12 ºC, 22 ºC and 22 ºC slurry conditions. Errors represent standard error of mean (SEM) of triplicate samples (n = 3)

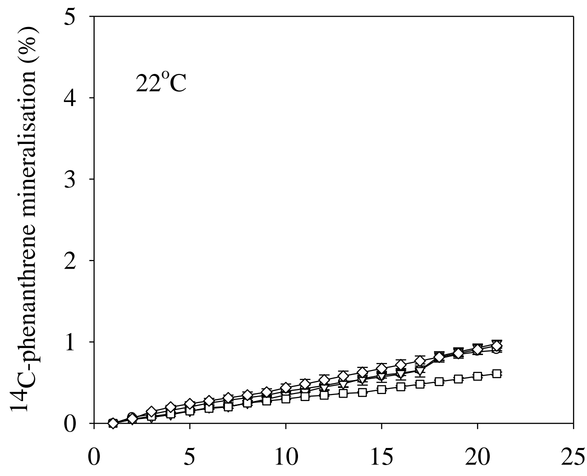
Soil-PAH contact (d)	**Soil**	**4 ºC (%)**	**12 ºC (%)**	**22 ºC (%)**	**Slurry (%)**
1	A	0.37 ± 0.1^{aC}	0.62 ± 0.0^{aC}	1.07 ± 0.1^{aB}	40.71 ± 1.8^{bB}
	B	0.46 ± 0.1^{aA}	0.87 ± 0.1^{bA}	2.17 ± 0.0^{cA}	28.01 ± 0.6^{aB}
	C	0.31 ± 0.0^{aA}	0.64 ± 0.0^{aA}	1.45 ± 0.2^{bA}	36.06 ± 5.2^{bA}
	D	0.36 ± 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}

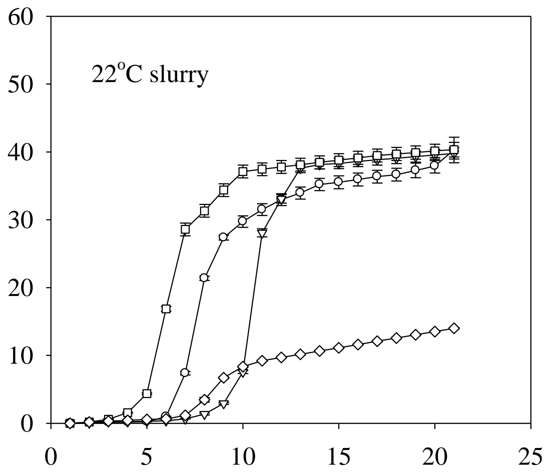
a: No statistical significant difference ($p > 0.05$) amongst soils within contact times; A: No statistical significant difference ($p > 0.05$) amongst same soils in different contact times; b, c or d: Statistical significant difference ($p < 0.05$) amongst soils within contact times; B, C or D: Statistical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

Soil A

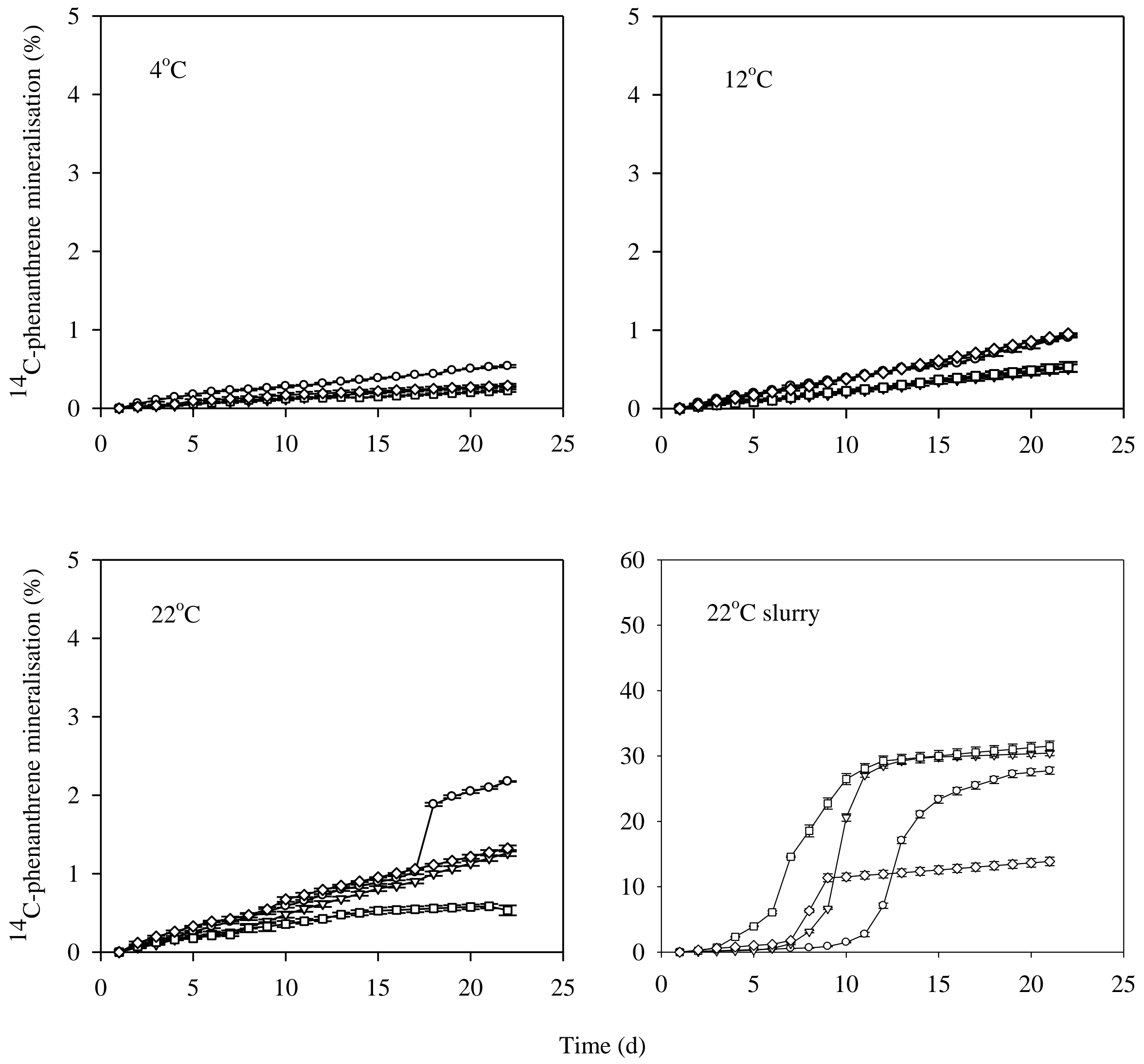




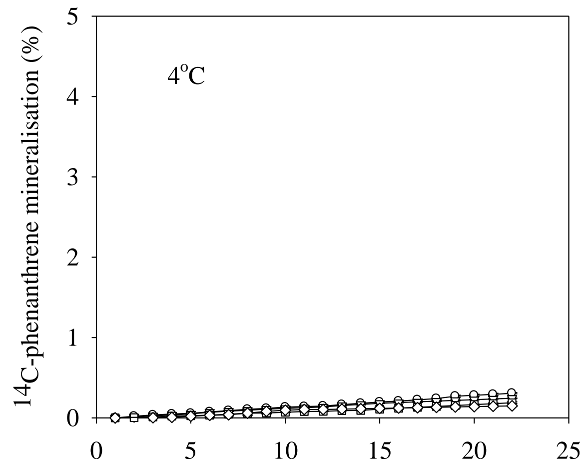


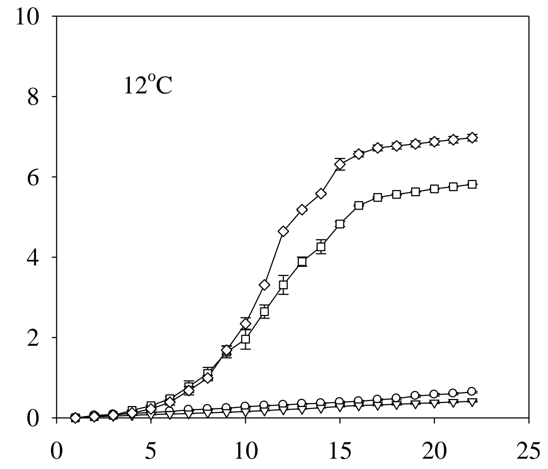


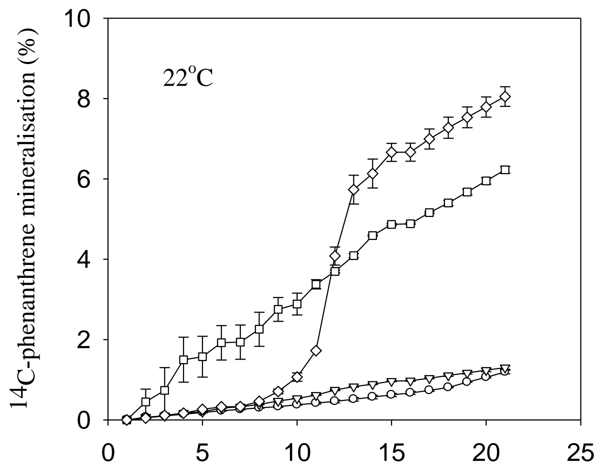
Time (d)

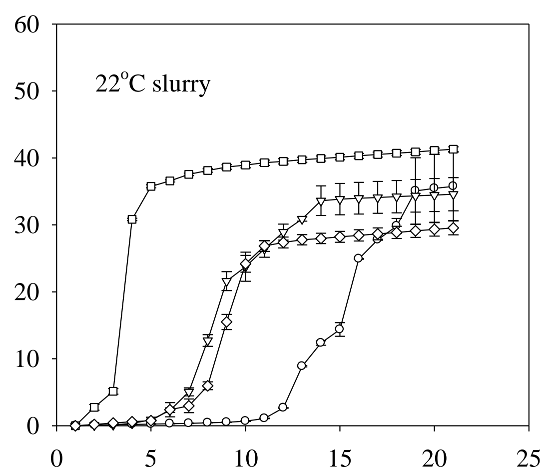

Soil B
^{14}C-phenanthrene mineralisation (%)
4°C
12°C
22°C
22°C slurry
0
5
10
15
20
25
0
1
2
3
4
5
0
10
20
30
40
50
60
Time (d)

Soil C









Time (d)

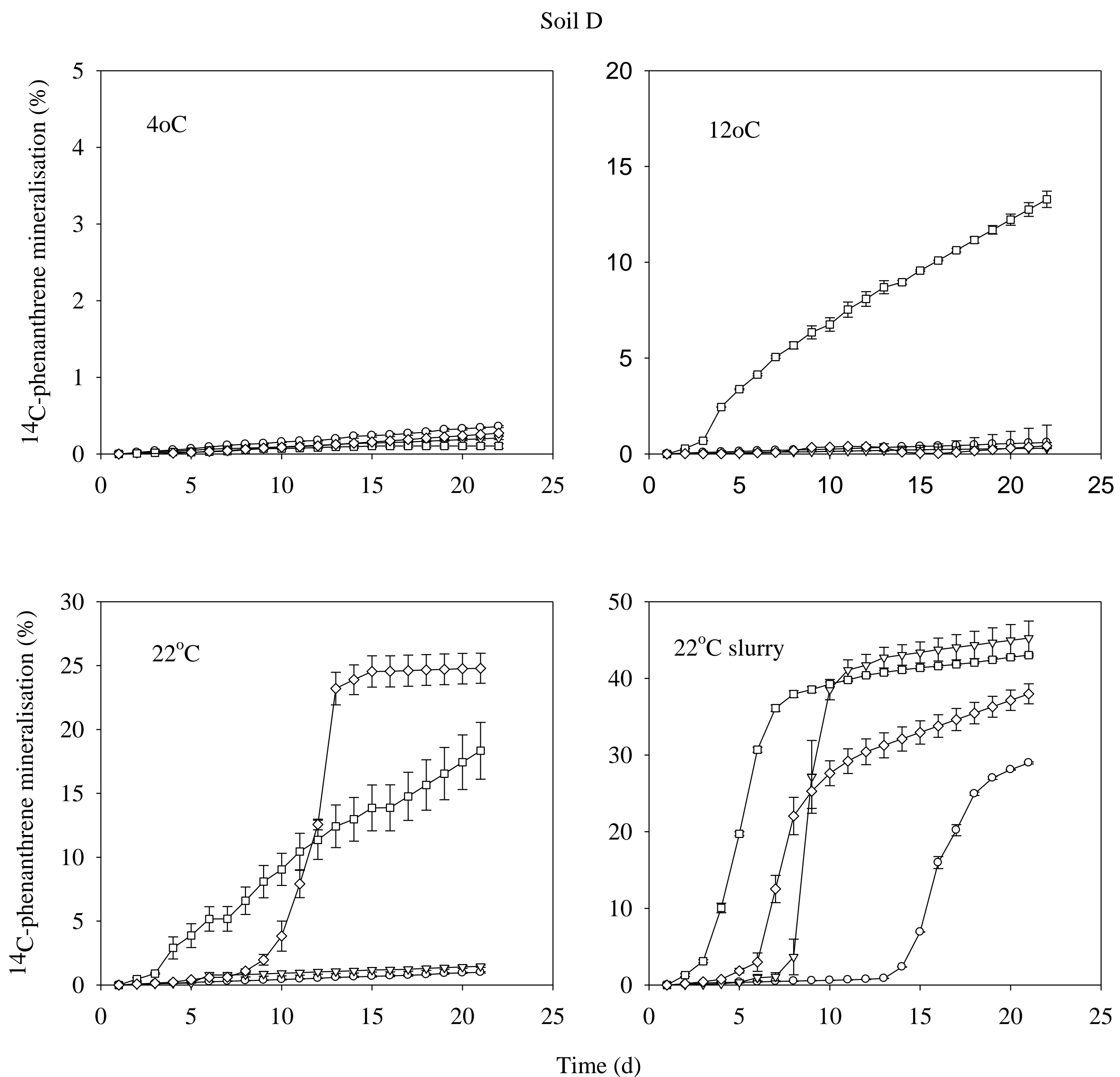
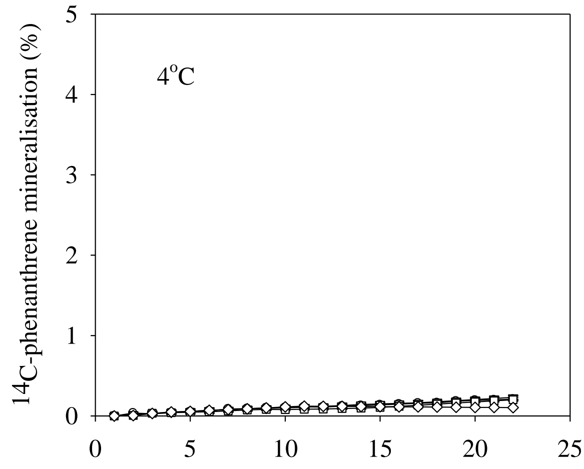
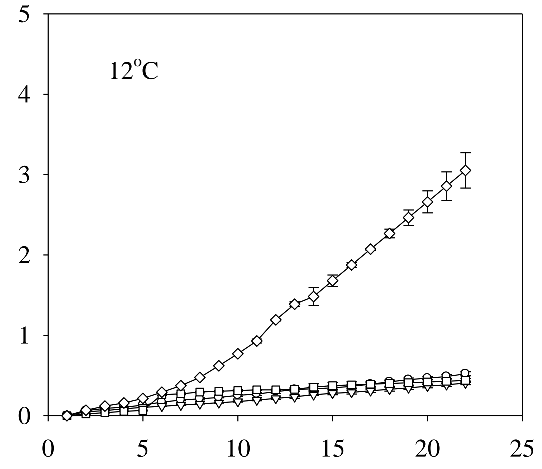
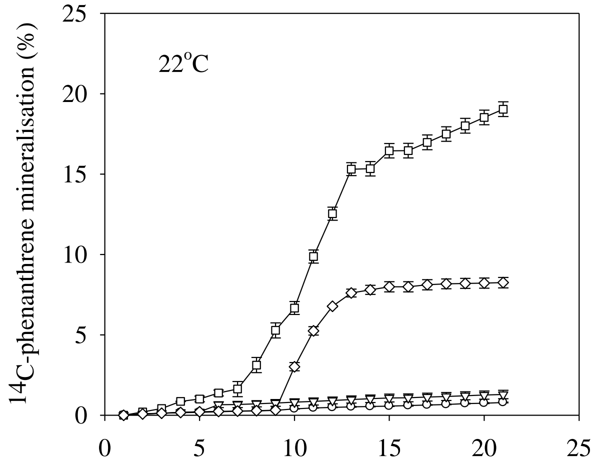


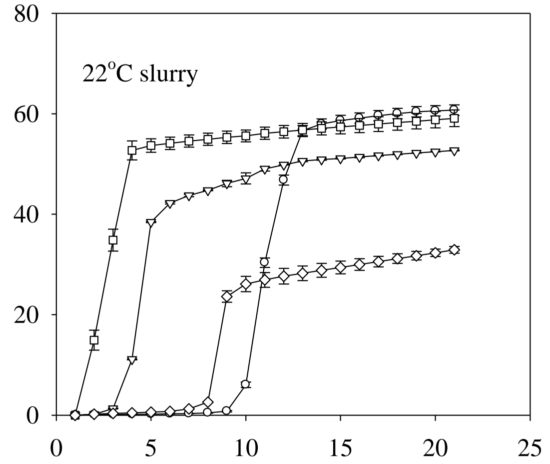
Figure 4 Mineralisation of ^{14}C-phenanthrene in soil D under different temperature regimes (4ºC, 12ºC, 22ºC, 22ºC slurry) after 1 (○), 30 (▽), 60 (□) and 150 (◊) days contact times. Error bars represent standard error of mineralisation (SEM) (n = 3).

Soil E









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).