The effect of organic acids on the behaviour and biodegradation of ^{14}C-phenanthrene in contaminated soil

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Abstract

The interaction between root exudates and soil microbes has been hypothesised as the primary mechanism for the biodegradation of organic pollutants in the rhizosphere. However, the mechanisms governing this loss process are not completely understood. This study aimed to investigate the effect of two important compounds within root exudates (citric and malic acid) on ^{14}C-phenanthrene desorption and bioaccessibility in soil. Overall results showed that the presence of both citric and malic acid (> 100 mmol l^{-1}) enhanced the desorption of ^{14}C-phenanthrene; this appeared to be concentration dependant. Increases in extractability were not reflected in a higher bioaccessibility. Despite enhancing the desorption of ^{14}C-phenanthrene in soil, there is no direct evidence indicating that citric or malic acid have the ability to promote the biodegradation of ^{14}C-phenanthrene from soil. Results from this study provide a novel understanding of the role that substrates, typically found within the rhizosphere due to root exudation, play in the bioaccessibility and biodegradation of hydrocarbons in contaminated soil.

Keywords: Phenanthrene, organic acids, root exudates, desorption, bioavailability, ageing

1 Introduction

The rhizosphere is defined as the soil in closest proximity to plant roots and has been hypothesised to enhance the biodegradation of organic contaminants such as aliphatic and aromatic hydrocarbons through different mechanisms (Anderson *et al.*, 1993; Pilon-Smits, 2005). These include the promotion of (1) larger microbial populations (Anderson *et al.*, 1993) and shifts on their community composition (Joner *et al.*, 2002), (2) source of biologically important substrates including nutrients and readily available sources of carbon (Reilley *et al.*, 1996; Dakora & Phillips, 2002; Martin *et al.*, 2014; Sivaram *et al.*, 2019), and (3) increasing the bioavailability of the contaminants due to root exudates, decay and turnover (Siciliano & Germida, 1998; Martin *et al.*, 2014; Sivaram *et al.*, 2019). The amount and type of substances released by roots is highly dependent on a series of factors including plant species and age, as well as particular soil and environmental conditions (Jones, 1998; Shukla *et al.*, 2011; Agnello *et al.*, 2014; Martin *et al.*, 2014). However, a number of low molecular weight compounds, such as amino acids, sugars and organic acids, have been identified as common constituents of root exudates (Jones *et al.*, 2003; van Hees *et al.*, 2005). Organic acids, including citric, malic and oxalic are reported to be the most abundant (Jones & Brassington, 1998; Ling *et al.*, 2015); therefore, it is reasonable to consider the role that these acids might play in influencing the extractability and the bioavailability of different contaminants and how this might influence their biodegradation.

The use of root exudates for the dissipation of organic contaminants in soil has been reported (Miya & Firestone, 2001; Yoshitomi & Shann, 2001; Joner *et al.*, 2002). These investigations have used simulated rhizosphere conditions by the introduction of artificial or natural root exudates in order to approach the subject in a more controlled manner (Miya & Firestone, 2001; Joner *et al.*, 2002). From these studies, research has been developed to consider the

effect of these substances on the shifts of the microbial populations and/or communities (Joner *et al.*, 2002; Shukla *et al.*, 2011), overall dissipation of contaminants (Joner *et al.*, 2002), and their effect on soil physical and chemical properties (Shukla *et al.*, 2011). Authors such as Sun *et al.* (2013), Martin *et al.* (2014) and Gao *et al.* (2015) have pointed out that although efforts have been directed towards investigating the effect of root exudates on the biodegradation of hydrocarbons in contaminated soil, information regarding the role of single compounds from this solution is scarce. Within these few studies, it has been reported that organic acids commonly found in root exudates can promote the desorption of phenanthrene from soil (Gao *et al.*, 2010b, 2015b; Ling *et al.*, 2015).

It has been observed that changes in the extractability of polycyclic aromatic hydrocarbons (PAHs) might act as a predictor of the microbial degradability of different species of PAHs. Specifically, the rates of desorption of some PAHs have successfully been used as predictors of their biodegradation (Cornelissen *et al.*, 1998a). As fractions of hydrocarbons are transferred from soil to solution through the desorption process; these can also become more bioaccessible and susceptible to be metabolised by the soil microbial community (Semple *et al.*, 2003, 2007, 2013). Therefore, the possibility of enhancing the desorption of PAHs by using organic acids to promote or enhance biodegradation in soil has been identified as a promising strategy, but remains poorly explored (Martin *et al.*, 2014). In addition, the extent into which these organic acids affect the biodegradation of the desorbed hydrocarbon has not been considered. Therefore, the aim of this study was to investigate the effect of two low molecular weight organic acids (LOAs) commonly found within root exudates in the extractability and bioaccessibility of ^{14}C-phenanthrene contaminated soil. Phenanthrene was selected as a model PAH given its widespread distribution, biodegradability and persistent properties in soil. For this, mineralisation, hydroxypropyl-β-cyclodextrin (HPCD) extractability and desorption kinetics of ^{14}C-phenanthrene were assessed in the presence of

85 organic acids at a range of concentrations. Results from this experiment provide a novel
86 perspective of the effect of organic acids on the fate of ^{14}C-phenanthrene soil by investigating
87 (1) the desorbing capacity of citric and malic acid and (2) the extent by which these can
88 promote a higher bioavailability and mineralisation.

89 **2 Methodology**

90 **2.1 Soil preparation and spiking**

91 An uncontaminated clay loam soil (top 20 cm, 2.7 % organic matter) was collected from
92 Myerscough Agricultural College, Preston, U.K. Partially air-dried soil (24 h) was passed
93 through a 2 mm sieve and stored in the dark at 4 ± 1 ºC until needed. Main soil physical and
94 chemical characteristics have been described by Towell *et al.* (2011). Sieved soil was
95 rehydrated (50% water holding capacity (whc)) and spiked following the procedure proposed
96 by Doick *et al.* (2003). In short, this approach consists on the application of the standards to a
97 fraction of the total amount of soil (inoculum) followed by gradual mixing and incorporation
98 of the remaining soil with a stainless steel spoon. Standards used for spiking contained $^{12/14}$C
99 phenanthrene dissolved in acetone to deliver a final concentration of 100 mg kg^{-1} (dw)
100 phenanthrene with an associated ^{14}C-activity of 83 Bq g^{-1} (dw). Spiked soil was placed in
101 sealed sterilized amber jars and incubated in the dark at 21 ± 1 ºC in a controlled environment
102 room for up to 15 weeks. Determination of the total ^{14}C-phenanthrene associated activity in
103 the soil was assessed at every time point by sample oxidation following the methodology
104 described by Rhodes *et al.* (2012).

105

2.2 Influence of organic acids on the mineralisation of ^{14}C-phenanthrene

Mycobacterium gilvum has been previously shown to degrade a range of hydrocarbons, including PAHs such as naphthalene, fluorine, phenanthrene, anthracene, fluoranthene and pyrene and as sole or primary source of carbon (Xiong *et al.*, 2017; Posada-Baquero *et al.*, 2019). In addition, this strain has also been isolated from chronically contaminated environments such as soil from a former coking plant (Xiong *et al.*, 2017), indicating its ability to adapt to these type of conditions, utilising hydrocarbons as their primary source of carbon under nutrient limiting conditions, which are typically observed on highly contaminated sites. To assess this, the mineralisation of ^{14}C-phenanthrene was measured using the methodology developed and tested by Reid *et al.* (2001) and Semple *et al.* (2006). Soil (10 g dw) aged over 14 and 50 days was placed into 250 ml modified Schott bottles fitted with a 1 M NaOH $^{14}CO_2$ trap (n = 3). These ageing times were selected based on that reported by Kelsey *et al.* (1997), where ^{14}C-phenanthrene mineralisation was significantly reduced within the first few weeks and then remained stable for 50 days. To assess the effect of organic acids towards the mineralisation process, citric, malic, oxalic and succinic acids were selected as representative LOAs often observed in the rhizosphere (van Hees *et al.*, 2005). Solutions containing individual LOAs within its naturally appearing range in the rhizosphere soil solution (0.1 and 0.5 mmol l^{-1}) (van Hees *et al.*, 2005) were used. These were incorporated into the minimal basal salts (MBS) medium and used for the mineralisation assay. For the mineralisation assays, soil was mixed with the MBS containing the organic acids (25 ml) and 5 ml of a bacterial inoculum of *M. gilvum* (10^5 cells ml^{-1}) to achieve a final 3:1 liquid:soil ratio. Bottles were then placed onto an orbital shaker at 100 rpm in a controlled environment room at 21 ± 1 ºC in the dark. $^{14}CO_2$ evolution was assessed by periodically (up to every 24 h) replacing the trap, mixing with 5 ml liquid scintillation cocktail and assessed by liquid scintillation counting (LSC) (10 min - Canberra Packard Tri-Carb 2300, U.K.).

2.3 Influence of organic acids on the extractability of ^{14}C-phenanthrene

2.3.1 Preliminary tests

A series of preliminary tests were carried in order to optimize the general experimental parameters and design of the extraction assays. Solutions of deionised water containing citric, malic, oxalic and succinic acids solutions of individual organic acids were prepared at 0.1 and 0.5 mmol l^{-1}. Desorption kinetics of ^{14}C-phenanthrene with these solutions (n = 3) were assessed from spiked soil following the methodology described below.

The temporal effect of organic acids on the bioaccessibility of ^{14}C-phenanthrene was also assessed (n = 3). Soil was saturated with malic acid solution (100 % whc) at two concentrations (0.5 and 500 mmol l^{-1}) and incubated in a controlled environment room 21 ± 1 ºC for 1, 3, 6, 8 or 24 h. Each experimental unit was also fitted with a 1 M NaOH $^{14}CO_2$ trap for the assessment of any possible dissipation of ^{14}C-phenanthrene by microbial respiration during the incubation time. Soil was extracted with 50 mM HPCD solutions after each incubation time following the methodology described below. $^{14}CO_2$ traps were assessed by adding 5 ml of liquid scintillation cocktail and assessed by LSC as previously described.

Citric, malic, oxalic and succinic acids did not impact significantly on the desorption of ^{14}C-phenanthrene from the soil (Table SI-1) when compared against the control ($p > 0.05$). Despite of this, soil extracted with citric and malic acid at 0.5 mmol l^{-1} were the only two treatments presenting higher rapidly desorbing fractions (F_{rap}), with (44.16 %) and (49.76 %) respectively, than the control (40.94 %). Based on this, these two organic acids were selected for further investigation at a wider range of concentrations (0.5, 100, 250, 500 and 1000 mmol l^{-1}) in order to assess the full potential of these compounds to impact the desorption of

^{14}C-phenanthrene in soil. Selected concentrations ranged from naturally appearing LOAs concentrations (van Hees *et al.*, 2005) up to maximum tested concentrations within experiments with similar aims (Gao *et al.*, 2015a; Ling *et al.*, 2015).

2.3.2 HPCD extraction of ^{14}C-phenanthrene from soil

Changes in the bioaccessibility of ^{14}C-phenanthrene were measured by HPCD extractions from soil aged over 1 and 15 weeks. At each time point, 1.25 g soil (dw) were placed into Teflon centrifuge tubes (n = 5); soil was saturated (100 % whc) with citric and malic acid solution (0.5, 100, 250, 500 and 1000 mmol l^{-1}). Sealed tubes where incubated in a controlled environment room (21 ± 1 ºC) for 8 h. Then, 25 ml of 50 mM HPCD solution was added. Tubes were placed onto an orbital shaker (100 rpm) for 22 h at 21 ±1 ºC. Afterwards, samples were centrifuged (3000 *x g* for 1 h) and 5 ml of the supernatant was placed in a glass scintillation vial and mixed with 15 ml liquid scintillation cocktail. Samples were assessed through LSC as described previously. Remaining ^{14}C-associated activity in the soil was assessed by sample oxidation (Rhodes *et al.*, 2012).

2.3.3 Desorption of ^{14}C-phenanthrene by organic acids

Tests were performed following a randomized design (n = 5) and blind sampling. Desorption kinetics were assessed after 1 and 15 weeks soil-contaminant contact time; at each time point, 4 g soil (dw) was placed into Teflon centrifuge tubes and mixed with 25 ml of organic acid solution at a given concentration (0.5, 100, 250, 500 and 1000 mmol l^{-1}). Tubes were placed onto an orbital shaker (100 rpm) in a controlled environment room at 21 ±1 ºC. Soil samples were sequentially extracted after 1, 4, 6, 12, 24, 45, 90, 180 and 360 h by centrifuging at 3000

177 *x g* for 1 h. Aliquots (5 ml) were mixed with 15 ml liquid scintillation cocktail in a glass
178 scintillation vial and assessed by LSC. Residual activity in the soil after the last extraction
179 was assessed by sample oxidation as described by Rhodes *et al.* (2012).

180 Desorption of ^{14}C-phenanthrene was examined by two- (Equation 1) and three-compartment
181 (Equation 2) first-order kinetics (Cornelissen *et al.*, 1998b; Rhodes *et al.*, 2010):

182 *Equation 1*:

183 $$S_t / S_0 = [F_{rap} \bullet \exp(-k_{rap} \bullet t)] + [F_{slow} \bullet \exp(-k_{slow} \bullet t)]$$

184 *Equation 2*:

185 $$S_t / S_0 = [F_{rap} \bullet \exp(-k_{rap} \bullet t)] + [F_{slow} \bullet \exp(-k_{slow} \bullet t)] + [F_{very\ slow} \bullet \exp(-k_{very\ slow} \bullet t)]$$

186 where S_t represents the amount of ^{14}C-phenanthrene sorbed to the soil at the desorption time t
187 (h) and S_0 is the initial total amount of ^{14}C-phenanthrene at the beginning of the assay (time
188 0). F_{rap}, F_{slow} and $F_{very\ slow}$ (%) are the rapid, slow and very slow desorbing fractions and k_{rap},
189 k_{slow} and $k_{very\ slow}$ (h^{-1}) are the rate constants for the rapid, slow and very slow desorption,
190 respectively. The model assumes that $k_{very\ slow} \leq k_{slow} \leq k_{rap}$ (Rhodes *et al.*, 2010; Clegg *et al.*,
191 2014), and that the addition of the desorbing fractions equals 100 % (Clegg *et al.*, 2014). The
192 values of F_{rap}, F_{slow}, $F_{very\ slow}$, k_{rap}, k_{slow} and $k_{very\ slow}$ were obtained by exponential curve
193 fitting using Excel Solver add-in, using a non-linear least squares method.

194

195 **2.4 Statistical analysis**

196 Statistical analyses were carried using the SPSS 21 (95 % confidence interval). Normality of
197 the data was verified by Shapiro-Wilk tests, transformations were applied in cases where a

198 normal distribution was not observed. Analyses of the differences across time were carried by
199 Student's t-test and Wilcoxon test for normal and not normally distributed data respectively.
200 Differences between the treatments at each time point were analysed using One-Way
201 ANOVA (Tukey) or Kruskal-Wallis test for normal and not-normal distributed data
202 respectively. Graphical representations of the results were done with the software Sigma Plot
203 2000.

204 **3 Results**

205 **3.1 Short-term impact of organic acids on the mineralisation of ^{14}C-phenanthrene in soil**

206 The impact of citric, malic, oxalic and succinic acids within a naturally occurring range of
207 concentrations was tested on the mineralisation of ^{14}C-phenanthrene. Organic acids were only
208 observed to produce significant differences on the mineralisation of ^{14}C-phenanthrene after
209 14 days soil-PAH contact time, while remaining unaffected after 50 days of soil-PAH contact
210 time. The data showed that after a short soil-PAH contact time (14 d), the presence of citric
211 acid (0.1 mmol l^{-1}) resulted in a significantly faster rate of mineralisation (29.52 % d^{-1}) than
212 the control (20.99 % d^{-1}) ($F = 2.795$, $p = 0.016$) (Table 1). At this same time point, although
213 not significant ($p = 0.077$), the lag phase of the control soil was longer (18.28 h) than in soil
214 incubated with organic acids (4.11 – 5.42 h).

215

216 **3.2 Preliminary tests for the selection of organic acids and soil-organic acid contact time**
217 **for the assessment of ^{14}C-phenanthre bioaccessibility in soil**

218 As significant differences were not observed within the different organic acids used in the
219 preliminary assay looking at their impact in ^{14}C-phenanthrene desorption kinetics; malic acid

was selected as a representative organic acid for the optimisation of the methodology for the assessment of bioaccessibility. Results from the test looking at the temporal impact of malic acid in HPCD-extractable ^{14}C-phenanthrene fraction showed that soil-organic acid contact time did not have a significant effect on the bioaccessible fraction of this hydrocarbon ($p > 0.05$). However, data showed that the largest extractable proportion of ^{14}C-phenanthrene was obtained after 8 h of soil-organic acid incubation (control soil, 8.15 %), compared to the lowest value presented after 48 hours (control soil, 1.10 %). Therefore, 8 h soil-organic acid incubation was considered to be the most suitable contact time and consequently selected for further investigation. Furthermore, mineralisation from the HPCD extractable experimental units within the incubation time was observed to be negligible.

3.3 Bioaccessibility of ^{14}C-phenanthrene in soil

Changes on the bioaccessibility of ^{14}C-phenanthrene in soil was assessed by HPCD extractions and were observed to be significantly different over time (Table 2, $t = 66.682$, $p < 0.001$). After one week of soil-PAH contact time; saturation of soil with organic acids (100 % whc, 8 h) did not have significant effects on the bioaccessibility of ^{14}C-phenanthrene ($F = 1.981$, $p = 0.059$). In the case of soil incubated for 15 weeks, the addition of 500 mmol l^{-1} citric acid significantly enhanced the bioaccessible fraction of ^{14}C-phenanthrene (14.92 %) compared to the control (6.72 %) ($F = 4.513$, $p = 0.003$).

3.4 Desorption of ^{14}C-phenanthrene with organic acids

The amount of ^{14}C-phenanthrene that was desorbed from soil was significantly affected by the presence of organic acids ($p < 0.001$) (Table 3). Citric acid at the highest concentration

(1000 mmol l^{-1}) consistently produced a significantly higher desorption than any other treatment after 1 week (39.27 %) and 15 weeks (47.86 %) soil-PAH contact time ($p < 0.001$). Furthermore, this was the only treatment capable of enhancing the desorption of ^{14}C-phenanthrene after 1 week soil-PAH contact time. The presence of citric acid (0.5 - 250 mmol l^{-1}) and malic acid (0.5 - 500 mmol l^{-1}) significantly reduced the total desorbable fraction of ^{14}C-phenanthrene soil after one-week soil-PAH contact time ($p < 0.001$). In contrast, only the lowest concentrations (0.5 mmol l^{-1}) of citric acid (13.04 %) and malic acid (12.51 %) produced significantly lower levels of desorption of ^{14}C-phenanthrene than the control (18.96 %) after 15 weeks soil-PAH contact time. This was in contrast to the desorption behaviour observed at concentrations above 100 mmol l^{-1} citric acid and 250 mmol l^{-1} malic acid, where desorbed ^{14}C-phenanthrene was significantly higher ($p < 0.001$).

3.4.1 Impact of organic acids on ^{14}C-phenanthrene desorption kinetics

Desorbing fractions (F_{rap} and F_{slow}; F_{rap}, F_{slow} and $F_{very\ slow}$) and rate constants (k_{rap} and k_{slow}; k_{rap}, k_{slow} and $k_{very\ slow}$) from the two- and three-compartment model fitting, respectively, are presented on Tables 4 and 5. Squared deviations data showed a better fit by the three-compartment one (Table SI-3, $p < 0.001$); therefore, further analysis was focused on the values estimated by this desorption model. Desorbing fractions (%) and rate constants (h^{-1}) obtained by the three-compartment model (Figures SI 4-9) showed significant differences for all cases ($p < 0.001$). After one-week soil-PAH contact time, significantly higher fractions of ^{14}C-phenanthrene were rapidly desorbed by 1000 mmol l^{-1} citric (19.22 %) and malic acid (20.20%) than in the control soil (12.08 %). In contrast, lower concentrations of malic acid (100 and 250 mmol l^{-1}) and citric acid (100 mmol l^{-1}) significantly reduced the rapidly desorbing fractions. Rapidly desorbing rate constants were not affected by the majority of the

267 treatments with the exception of the effect produced by citric acid at 100 mmol l^{-1}. Slowly
268 desorbing fractions were significantly reduced by all treatments apart from citric acid (1000
269 mmol l^{-1}), which was found to be similar to the control. Furthermore, rate constants of this
270 fraction (k_{slow}) were significantly enhanced in most of the treatments (except 0.5 and 1000
271 mmol l^{-1} citric acid), with a longest slowly desorbing phase produced in the present of malic
272 acid (0.139 – 0.146 h^{-1}) when compared against the control (0.013 h^{-1}). Very slowly
273 desorbing fractions accounted for the largest phase in all of the treatments. Moreover, organic
274 acids significantly increased this fraction in all treatments (except 1000 mmol l^{-1} citric acid),
275 ranging from 77.1 to 88.09 % against the 72.12 % when dH_2O was used as extractant. Very
276 slowly desorbing rate constants were also significantly higher in the presence of citric (≥ 500
277 mmol l^{-1}) and malic acid at all tested concentrations.

278 After 15 weeks incubation, high concentrations of citric (500 – 1000 mmol l^{-1}) and malic
279 (500 mmol l^{-1}) acid were found to significantly enhance the rapidly desorbing fraction of ^{14}C-
280 phenanthrene, representing up to 25.12 % compared to the control (13.11 %). Moreover, low
281 concentrations of both organic acids (0.5 mmol l^{-1}) had the opposite effect, significantly
282 reducing the fraction of ^{14}C-phenanthrene desorbed to 3.38 and 5.50 % respectively.
283 Similarly, rapidly desorbing rate constants were also significantly larger when soil was
284 extracted with citric (0.5 – 1000 mmol l^{-1}) and malic acid (1000 mmol l^{-1}). Slowly desorbing
285 fractions (F_{slow}) were significantly reduced by all tested concentrations of citric acid and 0.5,
286 100 and 1000 mmol l^{-1} malic acid, while the corresponding desorption rate constants
287 displayed the opposite behaviour. Fractions desorbed in the very slow phase were
288 significantly increased by all treatments (except 500 mmol l^{-1}malic acid) going from 3.23 %
289 in the control up to 92.50% when soil was treated with 0.5 mmol l^{-1}malic acid. Very slowly
290 desorbing rate constants were similar to the control with the exception of 100 mmol l^{-1}malic
291 acid where significantly higher values were observed ($p < 0.001$).

4 Discussion

4.1 Effect of organic acids in the bioaccessibility of ^{14}C-phenanthrene in soil

The bioaccessibility of PAHs can be quantified using different biological and chemical approaches. For the purposes of this study, the mineralisation and extraction of ^{14}C-phenanthrene by *M. gilvum* and HPCD were used, respectively. As a whole, these two methods are considered acceptable methodologies to assess not only the fraction of the hydrocarbon that is freely available to microorganisms, but also encompasses the fraction of the contaminant that may become bioavailable, and therefore removed from the soil (Semple *et al.*, 2004). The general absence of effects by organic acids on the mineralisation of ^{14}C-phenanthrene reported in this study has also been observed by Cébron *et al.* (2011) and Louvel *et al.* (2011), both of whom worked with root exudates containing mixtures of organic acids. Despite this trend, both authors were able to observe an initial acceleration of the mineralisation process (Cébron *et al.*, 2011; Louvel *et al.*, 2011), as was the case of citric acid (100 mmol l^{-1}) in this present study. Cébron *et al.* (2011) further discussed that this general absence of effects might be the consequence of enhanced sorption of phenanthrene to SOM and other soil inorganic fractions such as mineral clays caused by the organic acids, and that ultimately reflected in a reduction in the availability of phenanthrene for microbial degradation. Given the chemical characteristics of citric acid, other authors suggest that this LOA is capable of forming more stable complexes with different compounds in soil (An *et al.*, 2011; Ling *et al.*, 2015).

Similar trends were also observed when the bioaccessibility of ^{14}C-phenanthrene was measured through its HPCD extractability. Bioaccessibility was only significantly higher in one of the treatments (500 mmol l^{-1} citric and malic acid after 15 weeks soil-PAH contact time). These findings contrast with that reported by other authors where PAH availability can

316 be significantly promoted by different LOAs assessed through *n*-butanol extractions (Ling *et*
317 *al.*, 2009; Sun *et al.*, 2012, 2013; Kong *et al.*, 2013; Gao *et al.*, 2015b). Disagreement
318 between these two trends is suggested to be due to differences in the methodologies used for
319 this purpose, where *n*-butanol extracted PAH is not only the bioaccessible fraction of the
320 hydrocarbon, but also a portion of the non-bioaccessible PAH residues, as pointed by Ling *et*
321 *al.* (2009). Although *n*-butanol has been proposed to act as a predictor for the bioavailability
322 of PAHs in soil (Kelsey *et al.*, 1997; Liste & Alexander, 2002), this extractant has also been
323 observed to exhibit greater extraction efficiencies when compared against HPCD
324 extractability (Swindell & Reid, 2006). This has become important when comparing these
325 two methods to the mineralisation of phenanthrene in soil (Reid *et al.*, 2000; Rhodes *et al.*,
326 2010), where close linear 1:1 relationships have been observed for the case of HPCD
327 extracted PAH. Furthermore, *n*-butanol has also been demonstrated to act as a more
328 exhaustive extractant than HPCD (Reid *et al.*, 2000; Swindell & Reid, 2006), even extracting
329 similar quantities of PAHs than DCM, which is often use to determine total concentrations of
330 contaminants in soil (Reid *et al.*, 2000).

331 The impact that organic acids from the rhizosphere might have towards the biodegradation of
332 PAHs remains a poorly explored area; however, this is one of the presumed mechanisms by
333 which plant-enhanced bioremediation is thought to occur (Pilon-Smits, 2005). Changes in the
334 physico-chemcial conditions in soil, such as pH, may play an important role in the microbial
335 degradation of PAHs (Kästner *et al.*, 1998). Specifically, the acidic ranges of pH that the
336 presence of high concentrations of organic acids will produce, associated to the SOM-bound
337 PAHs that have been discussed, may be responsible to limit the microbial activity. This may
338 explain why, despite the fact that larger amounts of ^{14}C-phenanthrene can be extracted with
339 high concentrations of organic acids, the PAHs are not being biodegraded by soil bacteria and
340 metabolised to CO_2.

4.2 Impact of LOAs on the desorption of ^{14}C-phenanthrene in soil

The total desorbable fraction of ^{14}C-phenanthrene did not decrease as a function of time over the course of the incubation when soil was extracted with organic acids ($\geq$ 100 mmol l^{-1}). Despite the general acknowledgement of the negative correlation between the extractability of organic contaminants and contact time (Hatzinger & Alexander, 1995; Semple *et al.*, 2003), this behaviour was only observed in the control and the lowest tested concentration of organic acids after 15 weeks of soil-PAH contact time. This trend suggests that high amounts of organic acids could potentially restrict the reduction of bioaccessibility of ^{14}C-phenanthrene, therefore limiting the ageing process. Although not observed before, this behaviour could be the reflection of a dual effect of organic acids on phenanthrene sorption reported by Ouvrard *et al.* (2006) who described the impact of LOAs as a combined process characterised by an initial short term enhanced sorption of phenanthrene by SOM, followed by an increased mass transfer of the hydrocarbon due to the destabilisation of this soil fraction. Similarly, data from the present study showed a general reduction of the extractable ^{14}C-phenanthrene after a short period of ageing while organic acids were consistently observed to promote a larger desorption after 15 weeks of soil-PAH contact time when compared against the control. This increase in the extractability of PAHs by LOAs has also been reported by other authors (Ling *et al.*, 2009, 2015; Gao *et al.*, 2010a; b, 2015b; Kong *et al.*, 2013), but rarely considered the impact of soil-PAH ageing included in the present study. Although not common, the reduction of ^{14}C-phenanthrene desorption in the presence of organic acids observed after a short soil-PAH contact time in the present study has been reported before (Ouvrard *et al.*, 2006; Zhu *et al.*, 2009; Gao *et al.*, 2015b). This initial behaviour has been associated with the capacity of small amounts of oxalate, citrate and malate to promote the sorption of anions to the soil (Jones & Brassington, 1998; Jones *et al.*, 2003). In a similar way, phenanthrene has been hypothesised to be also sorbed through the

366 development of new sorption sites by these sorbed organic acids (Ouvrard *et al.*, 2006; Gao *et*
367 *al.*, 2015a).

368 LOAs have been acknowledged to significantly influence the physical, chemical and
369 biological properties of soil (Jones & Darrah, 1994; Jones, 1998). As such, the main
370 mechanism behind the enhancement of the desorption of PAHs in soil impacted by organic
371 acids has been proposed to be the solubilisation of soil organic matter (SOM) with a
372 subsequent release SOM-associated hydrocarbons (Ouvrard *et al.*, 2006; Agnello *et al.*,
373 2014). This explanation is supported by findings from different authors who have reported
374 consistently higher amounts of dissolved organic matter and certain minerals when artificial
375 root exudates (Gao *et al.*, 2010a) and single LOAs (Ling *et al.*, 2009, 2015; Gao *et al.*, 2010b,
376 2015a; Sun *et al.*, 2012; Kong *et al.*, 2013) were used to extract PAHs from contaminated
377 soil. In a similar way, previously immobilised aromatic compounds have been observed to be
378 released from soil to pore water after the introduction of organic acids solution (White *et al.*,
379 2003; Gao *et al.*, 2015b; Keiluweit *et al.*, 2015).

380 *4.2.1 Desorption kinetics*

381 ^{14}C-Phenanthrene desorption kinetics in the presence of the organic acids displayed a 3-
382 compartment desorption behaviour. Although desorption of organic contaminants has been
383 widely observed to behave with an initial rapid desorption followed by a slower phase
384 (Cornelissen *et al.*, 1998b), this rapid/slow/very slow release from the soil has also been
385 observed (Rhodes *et al.*, 2010). Typically, studies investigating desorption kinetics are
386 performed using extractants that are known (or suspected) to correlate with the biodegradable
387 fraction of the contaminant in question (Cornelissen *et al.*, 2001; Rhodes *et al.*, 2010).
388 However, this current research study was focused on the assessment of the desorbing
389 potential that organic acids might be able to provide. Bearing this in mind, the proportion of

390 ^{14}C-phenanthrene desorbed at each of these phases should be considered as a measure of the
391 behaviour of this PAH under the influence of organic acids rather than an indication of its
392 bioaccessibility.

393 The different fractions described by the desorption kinetics can be interpreted as the
394 biodegradable (F_{rap}), and less accessible F_{slow} and/or $F_{very\ slow}$ fractions of the organic
395 contaminant (Cornelissen *et al.*, 1998b; Rhodes *et al.*, 2010). Results from this investigation
396 showed that the majority of the treatments had a tendency to enhance the very slowly
397 desorbing fraction ($F_{very\ slow}$).These results could be interpreted as that the presence of organic
398 acids might be able to mobilise a significant proportion of the readily bioaccessible fraction
399 of ^{14}C-phenanthrene (F_{rap}) towards a less accessible form (F_{slow} and $F_{very\ slow}$), therefore
400 limiting the biological degradation of the contaminant or the rate at which this process takes
401 place (Pignatello & Xing, 1996; Clegg *et al.*, 2014). Moreover, similar behaviour has been
402 observed to occur during the mineralisation of organic acids, where these compounds have
403 been observed to induce shifts of $^{14}CO_2$ production from a rapid to a slower phase (Oburger
404 *et al.*, 2009).

405 **5 Conclusions**

406 Organic acids found within the rhizosphere play an important role on the behaviour of
407 phenanthrene in soil. It was found that the total extractable fraction of ^{14}C-phenanthrene can
408 be significantly enhanced by citric and malic acid. This effect is most likely to be observed at
409 a longer soil-PAH contact time, where organic acids showed to restrict the ageing effect.
410 Despite these enhancing effects, desorption kinetics indicated that the desorbed phenanthrene
411 was readily available given the behaviour as slow and very slow desorbing fractions. These
412 trends were confirmed when accessibility and mineralisation of ^{14}C-phenanthrene where
413 assessed. In this case, despite the enhancement of the total hydrocarbon extractable fractions

414 in the presence of citric and malic acid; there is no clear evidence suggesting that this
415 condition can promote the microbial utilisation of ^{14}C-phenanthrene. This study contributes to
416 the understanding of the role of root exudation within the rhizosphere towards the
417 bioaccessibility and biodegradation of hydrocarbons in contaminated soil. It is important to
418 note that organic acids may be able to remobilise contaminants, which were considered to be
419 non-bioaccessible. This may be important from a risk assessment perspective; however, the
420 concentrations of remobilised PAHs may be low and not represent a risk to environmental or
421 human health (Umeh et al., 2018).

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426

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Table 1. Mineralisation kinetics of ^{14}C-phenanthrene from soil affected by organic acids after 14 and 50 days ageing. Values of the lag phases (h), maximum rates (% d^{-1}) and total extents (%) represent the mean ± standard error of the mean (n = 3). Different letters indicate significant differences between the treatments at each time point assessed by post hoc Tukey tests.

		14 days ageing					
Treatment	Concentration (mmol l^{-1})	Lag phase*		Fastest rate		Total extent	
Control	0	18.288	± 0.358	^{a}20.991	± 0.564	^{ab}57.483	± 0.617
Citric acid	0.1	4.104	± 0.417	^{b}29.520	± 2.316	^{ab}65.325	± 3.590
	0.5	4.728	± 0.382	^{ab}25.519	± 1.439	^{ab}63.863	± 2.559
Malic acid	0.1	4.440	± 0.347	^{ab}27.114	± 1.268	^{ab}63.741	± 1.430
	0.5	4.320	± 0.364	^{ab}27.902	± 1.050	^{b}68.370	± 0.101
Oxalic acid	0.1	5.424	± 0.564	^{ab}22.562	± 2.125	^{a}54.085	± 3.386
	0.5	4.632	± 0.397	^{ab}26.093	± 1.576	^{ab}58.915	± 1.694
Succinic acid	0.1	4.656	± 0.260	^{ab}25.806	± 0.664	^{ab}63.543	± 2.409
	0.5	4.800	± 0.445	^{ab}25.270	± 1.938	^{ab}60.683	± 3.183
		50 days ageing					
Treatment	Concentration (mmol l^{-1})	Lag phase		Fastest rate		Total extent	
Control	0	^{a}84.350	± 2.400	^{a}1.613	± 0.257	^{a}11.581	± 1.321
Citric acid	0.1	^{a}81.128	± 1.945	^{a}1.607	± 0.128	^{a}11.419	± 0.560
	0.5	^{a}48.488	± 1.400	^{a}1.651	± 0.020	^{a}11.688	± 0.332
Malic acid	0.1	^{a}69.532	± 2.020	^{a}1.592	± 0.108	^{a}12.535	± 0.474
	0.5	^{a}85.519	± 2.319	^{a}1.345	± 0.089	^{a}10.590	± 0.667
Oxalic acid	0.1	^{a}69.922	± 0.894	^{a}1.553	± 0.026	^{a}12.387	± 0.410
	0.5	^{a}72.237	± 1.494	^{a}1.670	± 0.128	^{a}12.959	± 0.935
Succinic acid	0.1	^{a}78.770	± 1.819	^{a}1.661	± 0.140	^{a}11.093	± 0.591
	0.5	^{a}56.177	± 2.119	^{a}1.959	± 0.154	^{a}13.641	± 0.159

*Not normally distributed data analysed by Kruskal-Wallis non-parametric test (p = 0.077)

Table 2. HPCD extractable fraction of ^{14}C-phenanthrene from soil after 1 and 15 weeks ageing following saturation with organic acids solution (100 % whc, 8 h). Values represent the mean ± standard error of the mean (n = 5). Different letters indicate significant differences between the treatments at each time point (Tukey)

Treatment	Concentration (mmol l^{-1})	Bioaccessible ^{14}C-pheannthrene (%)	
		1 week*	15 weeks
Control	0	^{a}79.841 ± 0.717	^{ab}6.721 ± 0.970
Citric acid	0.5	^{a}78.964 ± 2.070	^{a}5.033 ± 0.955
	100	^{a}78.769 ± 1.346	^{ab}6.492 ± 1.646
	250	^{a}79.239 ± 1.243	^{abc}9.804 ± 1.033
	500	^{a}79.852 ± 0.633	^{c}14.929 ± 0.582
	1000	^{a}76.745 ± 0.164	^{bc}11.223 ± 1.524
Malic acid	0.5	^{a}81.138 ± 0.611	^{ab}7.992 ± 0.690
	100	^{a}80.517 ± 0.366	^{abc}8.938 ± 1.509
	250	^{a}78.540 ± 0.793	^{ab}8.764 ± 2.035
	500	^{a}77.937 ± 0.579	^{abc}10.726 ± 0.871
	1000	^{a}76.737 ± 1.003	^{ab}8.594 ± 1.284

Table 3. Total ^{14}C-phenanthrene desorbed from soil after 1 and 15 weeks ageing. Values represent the mean ± standard error of the mean (n = 5). Different letters indicate significant differences between the treatments at each time point (Tukey).

Treatment	Concentration (mmol l^{-1})	Desorbed ^{14}C-pheannthrene (%)	
		1 week	15 weeks
Control	0	^{de}27.980 ± 1.636	^{b}18.958 ± 0.931
Citric acid	0.5	^{ab}20.755 ± 0.432	^{a}13.038 ± 1.010
	100	^{a}17.221 ± 0.211	^{cd}26.462 ± 0.431
	250	^{ab}19.709 ± 0.081	^{de}31.264 ± 1.851
	500	^{cd}26.579 ± 0.795	^{f}40.006 ± 0.655
	1000	^{f}39.274 ± 1.921	^{g}47.856 ± 1.060
Malic acid	0.5	^{ab}20.068 ± 0.376	^{a}12.507 ± 0.176
	100	^{a}16.552 ± 0.119	^{bc}21.986 ± 1.363
	250	^{ab}18.820 ± 0.256	^{cd}25.923 ± 0.983
	500	^{bc}22.558 ± 0.266	^{e}32.955 ± 1.134
	1000	^{e}31.720 ± 1.249	^{ef}36.184 ± 2.054

Table 4. Desorbing fractions (F_{rap} and F_{slow}) and constant rates (k_{rap} and k_{slow}) calculated by a two-compartment model. Values represent the mean ± standard error of the mean (n = 5). Different letters indicate significant differences between the treatments assessed by post hoc Tukey tests

Treatment	Concentration (mmol l^{-1})	F_{rap} (%)		k_{rap} (h^{-1})		F_{slow} (%)		k_{slow} (h^{-1})	
1 week ageing									
Control	0	^{cd}16.571	± 0.442	^{a}0.128	± 0.004	^{cd}83.428	± 0.442	^{c}0.001	<0.001
Citric acid	0.5	^{abc}14.393	± 0.381	^{ab}0.142	± 0.004	^{def}85.606	± 0.381	^{ab}<0.001	<0.001
	100	^{a}12.313	± 0.160	^{b}0.145	± 0.001	^{f}87.687	± 0.160	^{a}<0.001	<0.001
	250	^{abc}14.423	± 0.108	^{ab}0.134	± 0.001	^{def}85.576	± 0.108	^{a}<0.001	<0.001
	500	^{bcd}19.090	± 0.706	^{ab}0.142	± 0.006	^{c}80.909	± 0.706	^{b}0.001	<0.001
	1000	^{e}28.381	± 1.587	^{ab}0.140	± 0.005	^{a}71.618	± 1.587	^{d}0.001	<0.001
Malic acid	0.5	^{abc}15.123	± 0.104	^{b}0.139	± 0.002	^{def}84.876	± 0.104	^{a}<0.001	<0.001
	100	^{a}11.901	± 0.143	^{ab}0.142	± 0.002	^{f}88.098	± 0.143	^{a}<0.001	<0.001
	250	^{ab}13.447	± 0.119	^{ab}0.146	± 0.002	^{ef}86.553	± 0.119	^{a}<0.001	<0.001
	500	^{d}16.122	± 0.272	^{ab}0.141	± 0.003	^{cde}83.877	± 0.272	^{ab}<0.001	<0.001
	1000	^{f}22.899	± 0.936	^{ab}0.140	± 0.003	^{b}77.100	± 0.936	^{c}0.001	<0.001
15 weeks ageing									
Control	0	^{b}14.683	± 3.591	^{ab}0.195	± 0.009	^{f}85.317	± 3.591	^{a}0.001	<0.001
Citric acid	0.5	^{a}6.789	± 0.247	^{ab}0.170	± 0.012	^{g}93.211	± 0.247	^{a}<0.001	<0.001
	100	^{bc}14.566	± 0.436	^{bc}0.217	± 0.020	^{ef}85.434	± 0.436	^{b}0.001	<0.001
	250	^{b}19.534	± 0.493	^{b}0.186	± 0.014	^{bc}80.466	± 0.493	^{bc}0.001	<0.001
	500	^{f}25.150	± 0.876	^{bc}0.213	± 0.009	^{c}74.850	± 0.876	^{cd}0.001	<0.001
	1000	^{g}30.907	± 0.377	^{c}0.262	± 0.006	^{a}69.093	± 0.377	^{d}0.002	<0.001
Malic acid	0.5	^{a}7.533	± 0.333	^{bc}0.206	± 0.023	^{g}92.467	± 0.333	^{a}<0.001	<0.001
	100	^{bcd}15.906	± 0.770	^{a}0.108	± 0.021	^{def}84.094	± 0.770	^{a}<0.001	<0.001
	250	^{cde}17.146	± 0.621	^{ab}0.156	± 0.021	^{cde}82.854	± 0.621	^{ab}0.001	<0.001
	500	^{e}20.412	± 0.598	^{bc}0.209	± 0.007	^{a}79.588	± 0.598	^{bc}0.001	<0.001
	1000	^{f}24.585	± 1.845	^{ab}0.156	± 0.009	^{b}75.415	± 1.845	^{bc}0.001	<0.001

Table 5. Desorbing fractions (F_{rap}, F_{slow} and $F_{very\ slow}$) and constant rates (k_{rap}, k_{slow} and $k_{very\ slow}$) calculated by a three-compartment model. Values represent the mean ± standard error of the mean (n = 5). Different letters indicate significant differences between the treatments assessed by post hoc Tukey tests.

Treatment	Concentration (mmol l^{-1})	F_{rap} (%)	k_{rap} (h^{-1})	F_{slow} (%)	k_{slow} (h^{-1})	$F_{very\ slow}$ (%)	$k_{very\ slow}$ (h^{-1})
1 week ageing							
Control	0	^{de}12.078 ± 0.418	^{ab}0.210 ± 0.008	^{e}15.801 ± 0.850	^{a}0.013 ± 0.001	^{b}72.122 ± 1.251	<^{a}0.001 <0.001
Citric acid	0.5	^{bcd}10.164 ± 0.564	^{b}0.226 ± 0.014	^{cd}9.162 ± 0.224	^{ab}0.021 ± 0.004	^{d}80.674 ± 0.708	<^{ab}0.001 <0.001
	100	^{a}7.099 ± 0.457	^{c}0.319 ± 0.037	^{b}7.136 ± 0.418	^{c}0.042 ± 0.003	^{efg}85.765 ± 0.224	<^{abc}0.001 <0.001
	250	^{b}8.984 ± 0.423	^{b}0.242 ± 0.013	^{bc}7.992 ± 0.219	^{bc}0.035 ± 0.003	^{de}83.025 ± 0.254	<^{abc}0.001 <0.001
	500	^{cde}11.822 ± 0.656	^{bc}0.271 ± 0.029	^{d}10.589 ± 0.536	^{c}0.036 ± 0.004	^{c}77.589 ± 0.563	<^{cde}0.001 <0.001
	1000	^{f}19.220 ± 0.344	^{b}0.234 ± 0.007	^{e}16.563 ± 0.614	^{abc}0.027 ± 0.005	^{a}64.217 ± 0.657	<^{cde}0.001 <0.001
Malic acid	0.5	^{cde}11.379 ± 0.524	^{a}0.139 ± 0.002	^{a}3.686 ± 0.473	^{d}0.139 ± 0.002	^{ef}84.935 ± 0.086	<^{de}0.001 <0.001
	100	^{bc}9.614 ± 0.136	^{a}0.142 ± 0.002	^{a}2.288 ± 0.008	^{d}0.142 ± 0.002	^{g}88.098 ± 0.143	<^{cde}0.001 <0.001
	250	^{bcd}11.074 ± 0.115	^{a}0.146 ± 0.002	^{a}2.373 ± 0.005	^{d}0.146 ± 0.002	^{fg}86.553 ± 0.119	<^{de}0.001 <0.001
	500	^{e}13.632 ± 0.260	^{a}0.141 ± 0.003	^{a}2.491 ± 0.012	^{d}0.141 ± 0.003	^{ef}83.877 ± 0.272	<^{e}0.001 <0.001
	1000	^{f}20.196 ± 0.914	^{a}0.140 ± 0.003	^{a}2.705 ± 0.022	^{d}0.140 ± 0.003	^{c}77.100 ± 0.936	<^{f}0.001 <0.001
15 weeks ageing							
Control	0	^{bcd}13.115 ± 0.712	^{b}0.172 ± 0.010	^{d}83.646 ± 3.300	<^{a}0.001 <0.001	^{a}3.239 ± 3.234	<^{a}0.001 <0.001
Citric acid	0.5	^{a}6.389 ± 0.180	^{bc}0.180 ± 0.019	^{c}14.050 ± 4.178	^{bc}0.005 ± 0.001	^{b}79.561 ± 4.036	<^{ab}0.001 <0.001
	100	^{bc}12.466 ± 0.512	^{cd}0.298 ± 0.023	^{c}16.386 ± 0.491	^{cd}0.010 ± 0.001	^{b}71.149 ± 0.609	<^{b}0.001 <0.001
	250	^{bc}11.753 ± 1.008	^{ef}0.511 ± 0.053	^{c}18.569 ± 1.781	^{de}0.027 ± 0.007	^{b}69.678 ± 2.345	<^{ab}0.001 <0.001
	500	^{ef}17.595 ± 1.768	^{de}0.521 ± 0.126	^{c}19.782 ± 1.110	^{d}0.020 ± 0.007	^{b}62.623 ± 2.536	<^{ab}0.001 <0.001
	1000	^{g}25.120 ± 1.153	^{de}0.398 ± 0.046	^{c}15.909 ± 2.951	^{de}0.026 ± 0.005	^{b}58.971 ± 2.787	^{ab}0.001 <0.001
Malic acid	0.5	^{a}5.504 ± 0.526	^{bc}0.190 ± 0.021	^{b}1.994 ± 0.305	^{f}0.190 ± 0.021	^{b}92.502 ± 0.324	<^{a}0.001 <0.001
	100	^{cde}14.762 ± 0.759	^{a}0.090 ± 0.012	^{a}1.057 ± 0.006	^{f}0.090 ± 0.012	^{b}84.181 ± 0.765	<^{a}0.001 <0.001
	250	^{def}16.916 ± 0.492	^{b}0.150 ± 0.010	^{d}58.727 ± 1.719	^{a}0.001 ± 0.000	^{b}24.357 ± 1.776	<^{a}0.001 <0.001
	500	^{f}20.465 ± 0.659	^{bc}0.210 ± 0.007	^{d}58.849 ± 1.998	^{ab}0.001 <0.001	^{ab}20.686 ± 1.732	^{a}0.001 <0.001
	1000	^{ab}9.066 ± 0.771	^{f}0.830 ± 0.032	^{c}18.118 ± 1.007	^{ef}0.061 ± 0.002	^{ef}72.816 ± 1.353	^{a}0.001 <0.001

Supplementary information

The effect of organic acids on the fate of ^{14}C-phenanthrene in contaminated soil

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Supplementary information

Table SI 1. Desorption kinetics of ^{14}C-phenanthrene from mildly aged soil (50 d). Values represent the mean ± standard error of the mean (n = 3). Different letters indicate significant differences between the treatments assessed by post hoc Tukey tests

Two-compartment fitting									
Treatment	Concentration (mmol l^{-1})	F_{rap} (%)		k_{rap} (h^{-1})		F_{slow} (%)		k_{slow} (h^{-1})	
Control	0	^{ab}40.939	± 3.861	^{a}0.090	± 0.019	^{ab}59.061	± 3.861	^{a}0.001	< 0.001
Citric acid	0.1	^{a}25.905	± 6.193	^{a}0.133	± 0.020	^{b}74.095	± 6.193	^{a}0.001	< 0.001
	0.5	^{ab}44.164	± 7.875	^{a}0.119	± 0.010	^{ab}55.836	± 7.875	^{a}0.001	< 0.001
Malic acid	0.1	^{a}25.356	± 3.052	^{a}0.179	± 0.007	^{b}74.644	± 3.052	^{a}0.001	< 0.001
	0.5	^{b}49.763	± 3.847	^{a}0.110	± 0.015	^{a}50.237	± 3.847	^{a}0.001	< 0.001
Oxalic acid	0.1	^{ab}37.096	± 5.009	^{a}0.107	± 0.017	^{ab}62.904	± 5.009	^{a}0.001	< 0.001
	0.5	^{ab}34.857	± 1.603	^{a}0.151	± 0.027	^{ab}65.143	± 1.603	^{a}0.001	± 0.001
Succinic acid	0.1	^{ab}30.451	± 2.869	^{a}0.115	± 0.036	^{ab}69.549	± 2.869	^{a}0.000	< 0.001
	0.5	^{ab}28.999	± 1.983	^{a}0.123	± 0.007	^{ab}71.001	± 1.983	^{a}0.001	< 0.001

Three-compartment fitting													
Treatment	Concentration (mmol l^{-1})	F_{rap} (%)		k_{rap} (h^{-1})		F_{slow} (%)		k_{slow} (h^{-1})		$F_{very\ slow}$ (%)		$k_{very\ slow}$ (h^{-1})	
Control	0	^{ab}36.112	± 5.067	^{ab}0.138	± 0.023	^{a}56.511	± 2.855	^{ab}0.003	< 0.001	^{a}7.377	± 2.458	^{a}0.003	< 0.001
Citric acid	0.1	^{a}22.920	± 6.498	^{ab}0.168	± 0.035	^{a}51.399	± 18.98	^{ab}0.003	± 0.001	^{a}25.682	± 13.80	^{a}0.002	± 0.001
	0.5	^{b}56.929	± 9.031	^{a}0.130	± 0.083	^{a}30.697	± 10.45	^{ab}0.007	± 0.005	^{a}12.374	± 12.37	^{a}0.001	± 0.001
Malic acid	0.1	^{a}23.902	± 2.529	^{ab}0.289	± 0.045	^{a}61.384	± 9.804	^{ab}0.002	± 0.001	^{a}14.714	± 8.010	^{a}0.001	± 0.001
	0.5	^{ab}43.770	± 2.514	^{ab}0.193	± 0.035	^{a}46.991	± 8.865	^{ab}0.005	± 0.003	^{a}9.240	± 6.792	^{a}0.002	± 0.001

Oxalic acid	0.1	[ab]31.645	± 7.967	[ab]0.321	± 0.179	[a]44.376	± 9.240	[ab]0.007	± 0.005	[a]23.979	± 15.78	[a]0.001	< 0.001
	0.5	[ab]29.134	± 1.809	[ab]0.375	± 0.082	[a]38.480	± 9.898	[ab]0.007	± 0.003	[a]32.386	± 10.93	[a]0.001	± 0.001
Succinic acid	0.1	[ab]29.880	± 2.748	[a]0.147	± 0.058	[a]64.678	± 5.535	[a]0.001	< 0.001	[a]5.443	± 4.019	[a]0.001	± 0.001
	0.5	[a]19.530	± 0.664	[b]0.577	± 0.106	[a]24.270	± 5.003	[b]0.016	± 0.006	[a]56.200	± 5.500	[a]< 0.001	< 0.001

Table SI 2. Proportion of ^{14}C-phenanthrene (1) extracted with 50 mM HPCD solution, (2) mineralisation rate (%, h^{-1}) within the assessed contact time. Values represent the mean ± standard error of the mean (n = 3)

Contact time (h)	Treatment	Extracted (%)[1]		Mineralised (%)[2]	
1	Control	7.269	± 0.993	1.454	± 0.199
	0.5 mmol l^{-1}	5.777	± 0.767	1.155	± 0.153
	500 mmol l^{-1}	7.854	± 0.257	1.571	± 0.051
3	Control	6.062	± 0.943	0.505	± 0.236
	0.5 mmol l^{-1}	6.101	± 0.358	0.508	± 0.089
	500 mmol l^{-1}	7.268	± 1.315	0.605	± 0.329
6	Control	5.843	± 1.035	0.243	± 0.259
	0.5 mmol l^{-1}	6.264	± 0.954	0.261	± 0.238
	500 mmol l^{-1}	5.528	± 0.818	0.230	± 0.205
8	Control	8.146	± 1.876	0.254	± 0.469
	0.5 mmol l^{-1}	5.978	± 1.104	0.186	± 0.276
	500 mmol l^{-1}	8.495	± 1.182	0.265	± 0.296
24	Control	5.250	± 3.213	0.054	± 0.803
	0.5 mmol l^{-1}	3.805	± 1.050	0.039	± 0.263
	500 mmol l^{-1}	6.282	± 1.327	0.065	± 0.332
48	Control	1.102	± 0.108	0.091	± 0.432
	0.5 mmol l^{-1}	0.929	± 0.169	0.077	± 0.676
	500 mmol l^{-1}	1.257	± 0.157	0.104	± 0.628

Table SI 3. Sums of squared deviations of desorbed ^{14}C-phenanthrene fitted to a two- and three-compartment model. Values represent the mean ± standard error of the mean (n = 5)

Treatment	Concentration (mmol l^{-1})	Sum of squared difference 2 compartment fitting		Sum of squared difference 3-compartment fitting	
		1 week ageing			
Control	0	9.05E-04	± 1.0E-04	2.24E-04	± 2.8E-05
Citric acid	0.5	4.79E-04	± 3.6E-05	2.53E-04	± 7.8E-05
	100	3.97E-04	± 2.6E-05	1.39E-04	± 1.3E-05
	250	4.19E-04	± 2.4E-05	1.28E-04	± 1.1E-05
	500	8.97E-04	± 1.0E-04	2.73E-04	± 5.1E-05
	1000	2.40E-03	± 2.8E-04	7.09E-04	± 9.4E-05
Malic acid	0.5	5.44E-04	± 4.0E-05	5.44E-04	± 4.0E-05
	100	3.13E-04	± 1.3E-05	3.13E-04	± 1.3E-05
	250	4.98E-04	± 2.0E-05	4.98E-04	± 2.0E-05
	500	6.71E-04	± 1.4E-05	6.71E-04	± 1.4E-05
	1000	1.49E-03	± 1.7E-04	1.49E-03	± 1.7E-04
		15 weeks ageing			
Control	0	4.50E-04	± 6.0E-05	4.60E-04	± 6.0E-05
Citric acid	0.5	9.59E-05	± 4.6E-05	6.00E-05	± 4.0E-05
	100	1.17E-03	± 2.0E-04	2.20E-04	± 9.3E-05
	250	2.50E-03	± 3.9E-04	1.48E-04	± 5.0E-05
	500	3.59E-03	± 4.5E-04	9.20E-04	± 1.9E-04
	1000	2.99E-03	± 7.8E-04	3.70E-04	± 4.8E-05
Malic acid	0.5	3.92E-04	± 6.4E-05	4.20E-04	± 6.6E-05
	100	4.63E-04	± 1.3E-04	4.63E-04	± 1.3E-04
	250	1.98E-03	± 1.2E-04	1.28E-03	± 4.1E-04
	500	1.75E-03	± 1.0E-04	1.58E-03	± 2.3E-04
	1000	3.70E-03	± 4.7E-04	2.80E-04	± 6.8E-05

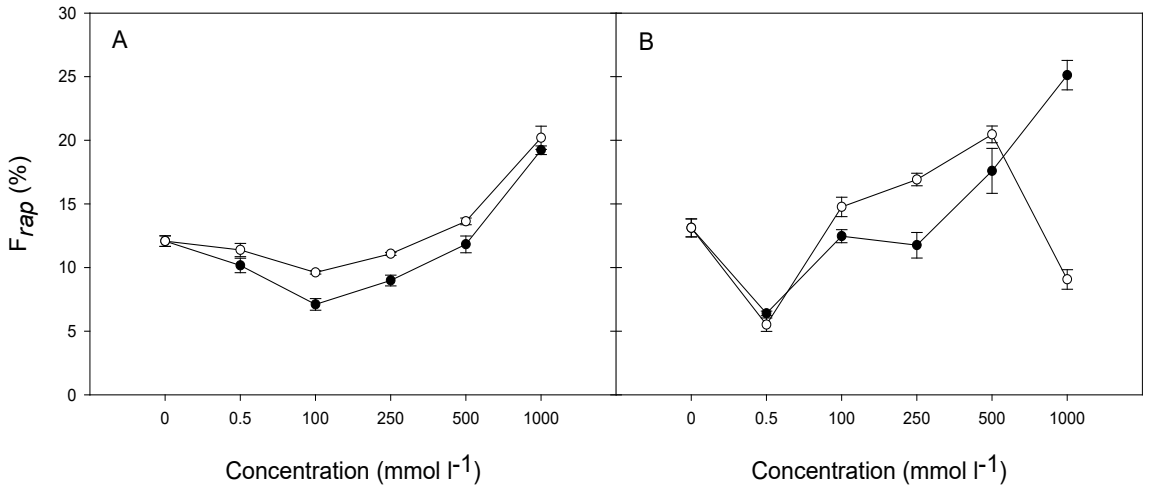

Figure SI 4. Rapid desorbing fractions (F_{rap}) of ^{14}C-phenanthrene from soil aged for 1 (A) and 15 (B) weeks extracted with citric (●) and malic (○) acid. Values were obtained using a three-compartment model fitting. Error bars represent the standard error of the mean (n = 5).

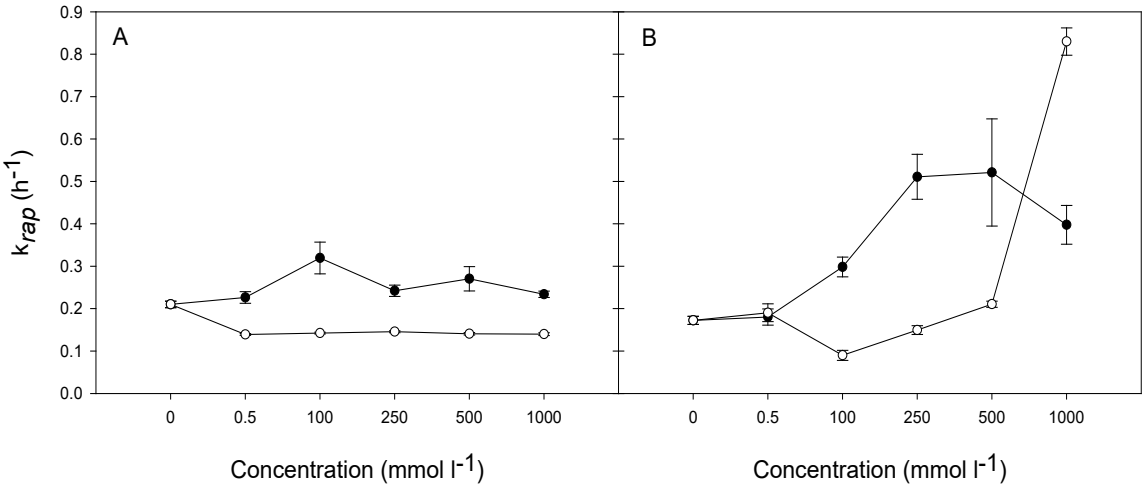

Figure SI 5. Rate constants of the rapid desorbing fractions (k_{rap}) of ^{14}C-phenanthrene from soil aged for 1 (A) and 15 (B) weeks extracted with citric (●) and malic (○) acid. Values were obtained using a three-compartment model fitting. Error bars represent the standard error of the mean (n = 5).

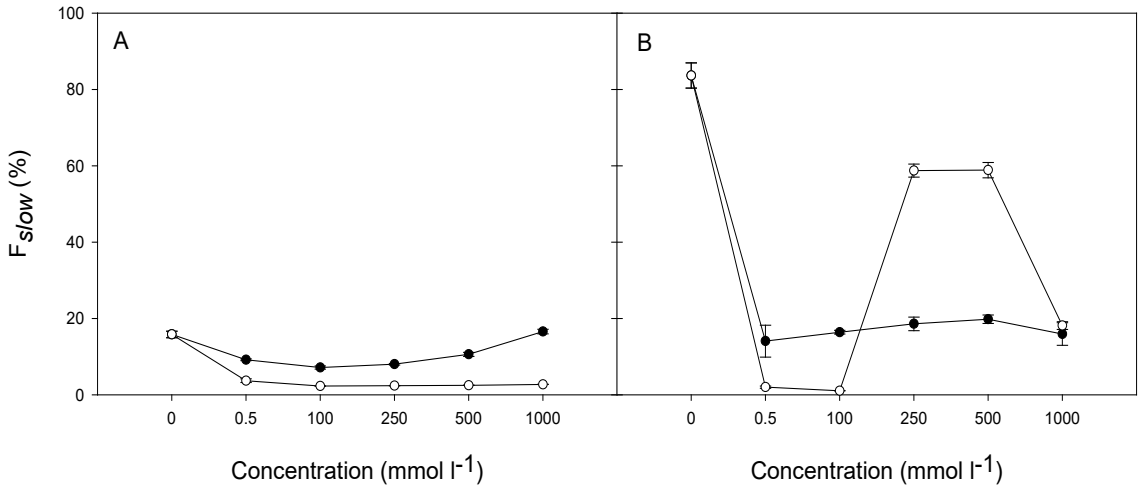

Figure SI 6. Slow desorbing fractions (F_{slow}) of ^{14}C-phenanthrene from soil aged for 1 (A) and 15 (B) weeks extracted with citric (●) and malic (○) acid. Values were obtained using a three-compartment model fitting. Error bars represent the standard error of the mean (n = 5).

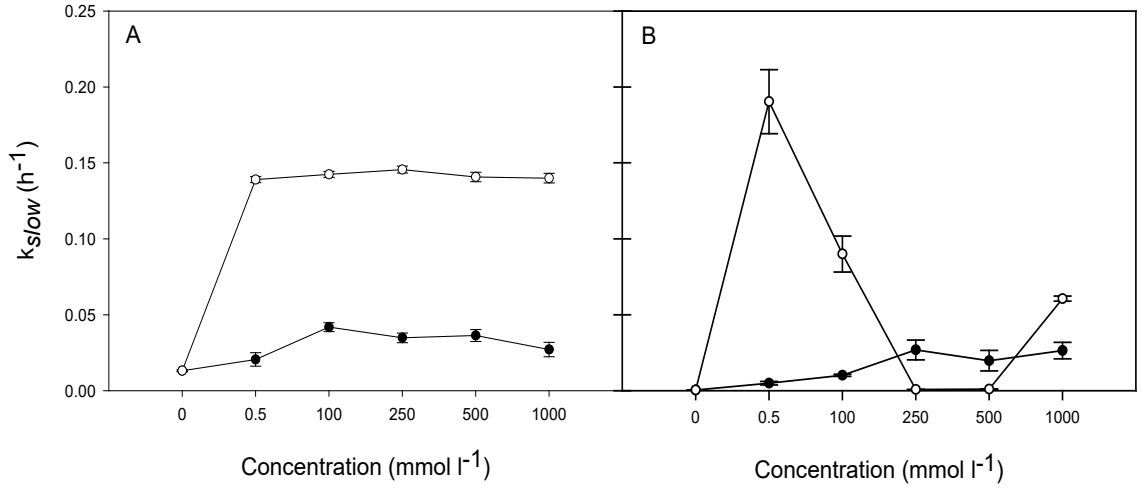

Figure SI 7. Rate constants of the slow desorbing fractions (k_{slow}) of ^{14}C-phenanthrene from soil aged for 1 (A) and 15 (B) weeks extracted with citric (●) and malic (○) acid. Values were obtained using a three-compartment model fitting. Error bars represent the standard error of the mean (n = 5).

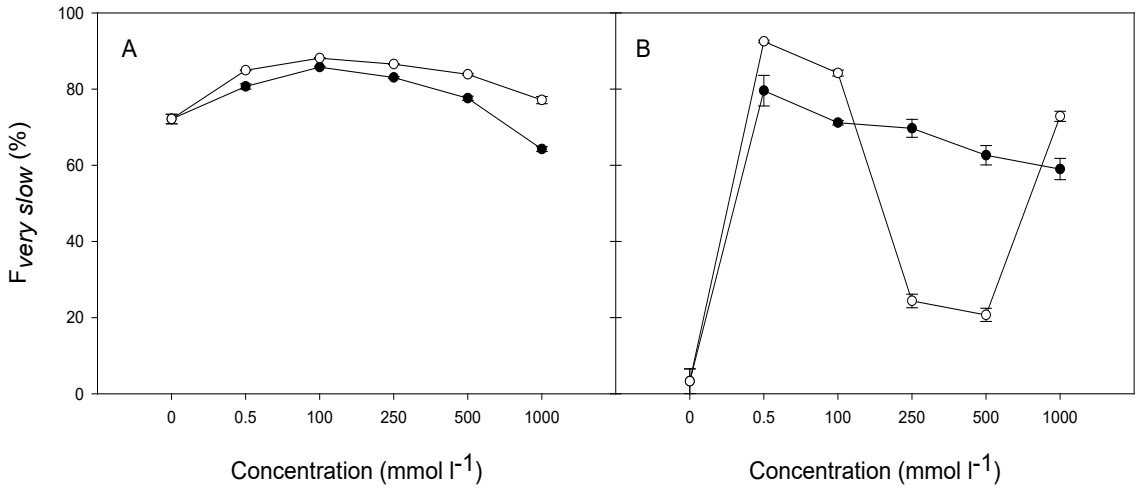

Figure SI 8. Very slow desorbing fractions ($F_{very\ slow}$) of ^{14}C-phenanthrene from soil aged for 1 (A) and 15 (B) weeks extracted with citric (●) and malic (○) acid. Values were obtained using a three-compartment model fitting. Error bars represent the standard error of the mean (n = 5).

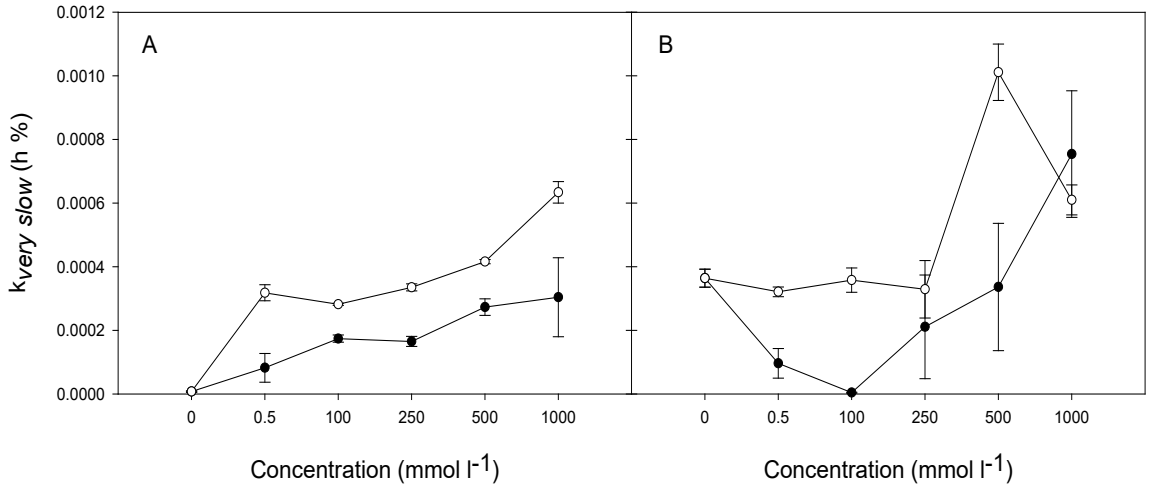

Figure SI 9. Rate constants of the very slow desorbing fractions ($k_{very\ slow}$) of ^{14}C-phenanthrene from soil aged for 1 (A) and 15 (B) weeks extracted with citric (●) and malic (○) acid. Values were obtained using a three-compartment model fitting. Error bars represent the standard error of the mean (n = 5).