Effect of lead, cadmium, and mercury co-contaminants on biodegradation in PAH-polluted soils

Michael E. Deary1 | Chinedu C. Ekumankama1 | Stephen P. Cummings2

1 Department of Geography and Environmental Sciences, Faculty of Engineering and Environment, Northumbria University, Ellison Building, Newcastle upon Tyne NE1 8ST, Tyne and Wear, UK
2 School of Science, Engineering & Design, Teesside University, Tees Valley, TS1 3BX, UK

Abstract
Contamination of land by persistent organic pollutants has significant implications for human health and for future development potential. Bioremediation is an effective method for reducing the concentrations of such contaminants to below harmful levels, but the presence of co-contaminants may hinder this process. Here, we present the results of a 40-week microcosm study in which the biodegradation of 16 United States Environmental Protection Agency (USEPA) polycyclic aromatic hydrocarbons (PAHs; total: 2,166 mg kg\(^{-1}\)) was followed in the presence of 3 different concentrations of cadmium (up to 620 mg kg\(^{-1}\)) and lead (up to 782 mg kg\(^{-1}\)) in a high organic matter soil. In the absence of metal treatment, 82% of PAHs were removed during the study period. Lead exerts a greater negative effect on total PAH removal than cadmium at low concentrations (approximately 100 mg kg\(^{-1}\)) whilst cadmium exerts the greatest effect at higher concentrations (up to \(-27.7\%\) reduction). Mercury, intended as the abiotic control (approximately 1,150 mg kg\(^{-1}\)), exerts the greatest effect overall (\(-37\%\)). Principal Component Analysis showed that PAH degradation was strongly associated with soil respiration rate, biomass content, and Ecoplate Average Well Colour Development. During the initial phase of the experiment, reduced microbial diversity was associated with increased PAH removal, consistent with literature observations for other organic contaminants, though this association was reversed after Week 12. Degradation of higher molecular weight PAHs showed the greatest sensitivity to the health of the microbial community. The effect of metal treatments on biotic parameters in microcosms without PAH amendment is also presented.

KEYWORDS
biodegradation, Biolog Ecoplate, cocontamination, PAHs, potentially toxic elements

1 | INTRODUCTION

Land degradation due to pollution from organic and inorganic contaminants is a significant issue, affecting both human health and the potential for future development and use (Chen et al., 2017, Roy & Mcdonald, 2015). In Europe alone, nearly three million sites are believed to be contaminated, with over 250,000 of these likely to need urgent remediation (European Commission, 2012; Jones et al., 2012). One class of organic contaminants, polycyclic aromatic hydrocarbons (PAHs), are persistent pollutants that are associated with...
many former industrial sites, particularly those with a history of coal gasification or waste incineration (Cerniglia, 1997). These toxic and in many cases, carcinogenic and mutagenic substances (Baird, Hooven, & Mahadevan, 2005) can be present in contaminated sites at concentrations of thousands of mg kg\(^{-1}\) and may therefore pose a significant environmental health risk. Remediation of such sites is often achieved using bioremediation (Gan, Lau, & Ng, 2009, Northcott & Jones, 2001, Wammer & Peters, 2005), which may be enhanced by bioaugmentation or biostimulation (Hamdi, Benzarti, Manusadzianas, Aoyama, & Jedidi, 2007; Straube et al., 2003; Tyagi, Da Fonseca, & De Carvalho, 2011). However, in assessing the viability of bioremediation, it is important to understand the effect that co-contaminants might have.

In the soil environment, PAHs distribute between the aqueous phase and multiple soil phases, each possessing a different degree of bioaccessibility (Deary, Ekumankama, & Cummings, 2016, Gramss, Voigt, & Kirsch, 1999, Hwang & Cutright, 2002, Johnsen, Wick, & Harms, 2005, Semple et al., 2004). In each of these phases, PAH removal involves a complex community of microorganisms that provide numerous enzymatic pathways for metabolism (Gramss et al., 1999; Johnsen et al., 2005), as well as producing biosurfactants that may solubilise the PAHs, thus increasing the rate and extent of biodegradation (Bezza & Chirwa, 2017, Hwang & Cutright, 2002, Johnsen et al., 2005). The soil microbial community also needs to be capable of metabolising the toxic intermediates of PAH degradation (Baboshin & Golovleva, 2012). For all these reasons, it might be thought that increased microbial diversity should be associated with more extensive PAH degradation. Moreover, any factor affecting microbial diversity, such as the presence of metal co-contaminants, as in this study, might be expected to impair the overall extent of biodegradation. Nevertheless, literature studies have often reported the opposite, that is, that reduced microbial biodiversity is associated with positive effects on biodegradation, for example, soils contaminated with diesel (Bell, Yergeau, Juck, Whyte, & Greer, 2013; Jung, Philippot, & Park, 2016). This relationship has been explained in terms of a reduced competitive inhibitive effect on the key biodegrading species, that is, through the removal of species that are utilising soil resources or colonising strategic soil environments yet are not directly contributing to the biodegradation process (Bell et al., 2013, Jung et al., 2016).

It is therefore important to investigate how microbial biodiversity and other biotic parameters are related to the scope and efficiency of PAH removal in soils, especially since PAH-polluted sites are often cocontaminated with heavy metals at concentrations likely to impair the microbial community structure (Atagana, 2006). In the present paper, we report on the effect that cadmium (Cd; up to 620 mg kg\(^{-1}\)) and lead (Pb; up to 782 mg kg\(^{-1}\)) amendment has on the biodegradation of PAHs during a 40-week period, in microcosms containing an urban greenfield soil of high organic carbon content (11%). Similar Pb concentrations to ours have been used in previous studies that have looked at the impact of metals on PAH biodegradation (Maliszewska-Kordybach & Smreczak, 2003, Thavamani, Malik, Beer, Megharaj, & Naidu, 2012). Cd concentrations reported in these same studies were somewhat lower (up to 112 mg kg\(^{-1}\)) than used in the present study; however, in choosing our concentration range, we felt it was important to compare relative effects for similar concentrations of Pb and Cd.

Principal Component Analysis (PCA) was used to relate the extent of PAH degradation (16 individual USEPA PAHs), in the metal-amended soils, to trends in simultaneously measured biotic parameters that are indicative of the health and diversity of soil microbial communities. The biotic parameters studied were (a) the diversity of microbial species present (through Ecoplate community level physiological profiling and the calculation of the Shannon Diversity Index, H), (b) the amount of biomass present (soil microbial biomass carbon concentration, C\(_{\text{mic}}\)), (c) the relative metabolic rate of the active microbial community (soil respiration rate), and (d) the degree of environmental stress to which the microbial community is subject (soil metabolic quotient, qCO\(_2\)). The effect of Cd, Pb, and mercury (Hg) on control microcosms, containing no PAHs, was also studied.

## METHODOLOGY

### 2.1 Overview

Full details of the experimental set-up, including PAH- and metal-spiking procedures, can be found in Deary et al. (2016). In summary, the experiment was carried out over a 40-week period in soil microcosms cut from white Polyvinyl Chloride (PVC) piping of drinking-water standard (30 cm long and 3.2 cm diameter). PAH and metal spiking of the soil was carried out in bulk in a cement mixer. For each separate treatment, 42 microcosms were prepared, each containing approximately 250 g wet weight of soil; this allowed the harvesting of three replicates of each treatment for analysis at 0, 1, 2, 3, 5, 7, 9, 12, 15, 20, 25, 30, 35, and 40 weeks. The microcosms were stored at 20 °C with diurnal light cycle and were maintained at 75% of the maximum water holding capacity. The soil used for these studies was sampled from a wooded urban greenfield site in Newcastle upon Tyne, UK. The soil contained 11.4% organic carbon, 0.37% nitrogen (both measured using Accelerated Solvent Extraction and analysed for the 16 USEPA PAHs using Gas Chromatography with Mass Spectrometry (GC–MS)), as described in Deary et al. (2016).

### 2.2 PAH degradation

For each of the soil treatments, triplicate microcosms were extracted using Accelerated Solvent Extraction and analysed for the 16 USEPA PAHs using Gas Chromatography with Mass Spectrometry (GC–MS), along with other soil properties. Hg was used to sterilise soil microcosms that were to serve as abiotic controls; however, some biological activity returned after the initial spiking, and the procedure had to be repeated at 7 weeks. The total PAH concentration for amended samples was 2,166 mg kg\(^{-1}\) with individual abundances detailed in Table 2.
### TABLE 1  Details of metal spiking treatments

| PAH amendment status | Metal amendment | Metal amendment level | Designation used in graphs and tables | Initial concentration (mg kg\(^{-1}\) ± 1 SD on a dry weight basis) |
|----------------------|-----------------|-----------------------|---------------------------------------|---------------------------------------------------------------|
| Non amended          | Cadmium         | None                  | None                                  | <1                                                            |
|                      |                 | Low                   | Cd:L                                  | 149 ± 6                                                       |
|                      |                 | Medium                | Cd:M                                  | 319 ± 11                                                      |
|                      |                 | High                  | Cd:H                                  | 567 ± 48                                                      |
| PAH amended          | Cadmium         | None                  | None\(^a\)                            | <1                                                            |
|                      |                 | Low                   | Cd:L\(^a\)                            | 134 ± 21                                                      |
|                      |                 | Medium                | Cd:M\(^a\)                            | 302 ± 13                                                      |
|                      |                 | High                  | Cd:H\(^a\)                            | 620 ± 69                                                      |
| Non amended          | Lead            | None                  | None                                  | 207 ± 9                                                       |
|                      |                 | Low                   | Pb:L                                  | 340 ± 8                                                       |
|                      |                 | Medium                | Pb:M                                  | 566 ± 43                                                      |
|                      |                 | High                  | Pb:H                                  | 817 ± 16                                                      |
| PAH amended          | Lead            | None                  | None\(^a\)                            | 210 ± 12                                                      |
|                      |                 | Low                   | Pb:L\(^a\)                            | 339 ± 36                                                      |
|                      |                 | Medium                | Pb:M\(^a\)                            | 523 ± 3                                                       |
|                      |                 | High                  | Pb:H\(^a\)                            | 782 ± 45                                                      |
| Non amended          | Mercury         | N/A                   | Hg                                    | 643 ± 27\(^b\)                                               |
| PAH amended          | Mercury         | N/A                   | Hg\(^a\)                              | 627 ± 20\(^c\)                                               |

Note: PAH = polycyclic aromatic hydrocarbons.
\(^a\)indicates PAH-amended treatments.
\(^b\)respiked at same concentration after Week 5 to give a concentration of approximately 1,142 mg kg\(^{-1}\).
\(^c\)respiked at same concentration after Week 5 to give a concentration of approximately 1,115 mg kg\(^{-1}\).

### TABLE 2  Percentage reduction in extractable concentration of the 16 USEPA PAHs compared with the control from soil microcosms over a 40-week period under different metal treatment conditions. Metal concentrations are given in Table 1

| PAH                  | No. rings in PAH structure | Relative abundance of PAH at 0 days | Percentage removal in absence of metals | Percentage reduction in extractable PAH concentration for different metal treatments |
|----------------------|-----------------------------|-------------------------------------|----------------------------------------|-------------------------------------------------------------------------------------|
|                      |                             |                                     |                                        | Pb:L  | Pb:M  | Pb:H  | Cd:L  | Cd:M  | Cd:H  | Hg      |
| Naphthalene          | 2                           | 0.2                                 | 82.1                                   | 13.2  | 14.2  | 14.6  | 3.1   | 11.2  | 13.7  | 16.4   |
| Acenaphthylene       | 3\(^a\)                     | 0.1                                 | 89.0                                   | 8.4   | 11.9  | 13.8  | 7.7   | 8.9   | 10.4  | 16.0   |
| Acenaphthene         | 3\(^a\)                     | 0.4                                 | 96.3                                   | 2.3   | 2.3   | 2.7   | 1.3   | 0.4   | 1.7   | 3.7    |
| Fluorene             | 3\(^a\)                     | 0.4                                 | 96.9                                   | 3.3   | 2.9   | 3.4   | 1.9   | 3.1   | 4.1   | 4.9    |
| Phenanthrene         | 3                           | 4.4                                 | 98.6                                   | 2.4   | 2.5   | 2.6   | 1.5   | 3.1   | 4.4   | 16.4   |
| Anthracene           | 3                           | 1.1                                 | 95.2                                   | 1.8   | 5.5   | 7.8   | 1.2   | 7.1   | 11.7  | 19.0   |
| Fluoranthene         | 4\(^a\)                     | 9.7                                 | 94.9                                   | 0.9   | 1.0   | 2.2   | 0.7   | 2.3   | 8.0   | 30.1   |
| Pyrene               | 4                           | 8.9                                 | 95.0                                   | 0.7   | 0.8   | 2.5   | 2.4   | 2.5   | 3.5   | 16.3   |
| Benzo[a]anthracene   | 4                           | 7.0                                 | 84.4                                   | 5.6   | 7.6   | 9.0   | 5.1   | 15.1  | 28.4  | 31.1   |
| Chrysene             | 4                           | 9.1                                 | 83.4                                   | 11.0  | 16.2  | 18.1  | 10.8  | 9.0   | 16.8  | 19.5   |
| Benzo[b]fluoranthene | 5\(^a\)                     | 15.3                                | 69.6                                   | 19.0  | 11.6  | 24.1  | 2.5   | 24.1  | 33.4  | 31.2   |
| Benzo[k]fluoranthene | 5\(^a\)                     | 6.4                                 | 76.3                                   | 20.0  | 29.3  | 37.0  | 10.1  | 24.7  | 39.7  | 49.2   |
| Benzo[a]pyrene       | 5                           | 11.8                                | 75.8                                   | 24.9  | 26.4  | 32.3  | 3.7   | 28.6  | 41.0  | 48.6   |
| Indeno(cd-123)pyrene | 6\(^a\)                     | 11.7                                | 81.5                                   | 25.0  | 34.0  | 36.6  | 10.9  | 37.9  | 43.4  | 54.7   |
| Dibenzo[ah]anthracene| 5                           | 4.8                                 | 72.9                                   | 12.8  | 16.5  | 28.7  | 4.9   | 20.7  | 49.9  | 64.6   |
| Benzo[ghi]perylene   | 6                           | 8.7                                 | 81.7                                   | 23.4  | 34.7  | 41.0  | 5.3   | 21.6  | 48.9  | 68.7   |
| Total PAH            | n/a                         | 100.0                               | 82.2                                   | 13.4  | 16.3  | 20.7  | 4.9   | 17.6  | 27.7  | 37.6   |

Note: PAH = polycyclic aromatic hydrocarbons.
\(^a\)nonalternant PAHs.
2.3 | Soil respiration

Soil respiration rate was determined using the OxiTop manometric system (Kaakinen, Vahaoja, Kuokkanen, & Roppola, 2007, Platen & Wirtz, 1999, Schaefer & Juliane, 2007, The International Organization for Standardization, 2002). A combined soil sample was obtained from triplicate microcosms. Maintained at 75% water holding capacity, 100 g of soil was transferred to a 250 mL OxiTop bottle and incubated at 20 °C ± 1 for 2 days.

The soil respiration rate, \( BA \) (mg O\(_2\) kg\(^{-1}\) d\(^{-1}\)) was calculated from the linear region of the pressure decrease curve using Equation (1) (Platen & Wirtz, 1999), where \( M_o(O_2) \) = molar mass of oxygen (32,000 mg mol\(^{-1}\)); \( V_f \) = free gas volume (0.195 L); \( R \) = gas constant (83.14 L mb mol\(^{-1}\) K\(^{-1}\)); \( T \) = measurement temperature (293.15 K); \( m_{dt} \) = mass of dry soil (kg); and \( \Delta p \) = pressure reduction (mb d\(^{-1}\)).

\[
BA = \frac{M_o(O_2)}{R T} V_f \frac{\Delta p}{m_{dt}} \tag{1}
\]

2.4 | Community-level physiological profiling using BIOLOG Ecoplates

BIOLOG Ecoplates are an established, rapid, and reproducible technique for measuring functional diversity of the microbial community (Choi & Dobbs, 1999, Kirk et al., 2004) that has been applied in numerous similar studies (Derry, Staddon, & Trevors, 1998; Kumar, Shah, & Walker, 2011; Niklinska, Chodak, & Laskowski, 2005; Sun et al., 2010; Sun et al., 2013). Preston-Mafham, Boddy, and Randerson (2002) and Weber & Legge (2010) have reviewed the practicalities and potential problems in the use and interpretation of community-level physiological profiling.

Each BIOLOG Ecoplate contains 96 wells, comprising three replicates of 31 separate sole carbon substrates, together with three blank wells. We broadly followed the method of Niklinska et al. (2005), having first established, in a series of test runs, a suitable inoculum dilution, and plate incubation period. Three grams of soil was placed in a 50 mL tube and made up to 30 mL with 0.9% (w/v) NaCl solution. The mixture was gently mixed with an end-over rotary shaker (Stuart Rotator Shaker SB3) for 60 min at 30 rpm and room temperature. The solution was centrifuged (Beckman Allegra 6R Centrifuge) at 200 rpm for 5 min (Kirk, Klironomos, Lee, & Trevors, 2005), and the supernatant was diluted 50 fold using 0.9% NaCl solution; 150 μL of this was used to inoculate each well. For each of the soil treatments, triplicate microcosms were analysed on one Ecoplate.

The inoculated plates were incubated at 22 °C for 50 hr. Colour development in the wells was measured at 595 nm using an ELx808 absorbance microplate reader (BioTek Instruments Inc.). Absorbance values were blanked against the control well (inoculated but without a sole carbon source). Average relative standard deviations for triplicate microcosms across all treatments and weeks (excluding the Hg results) were 10.0% for non-PAH-amended samples and 9.2% for PAH-amended samples. Visualisations of the plate absorbance data across 40 weeks for each of the 31 substrates are shown in Figures S1–S10.

2.5 | Analysis of Ecoplate data

Average Well Colour Development (AWCD), a widely used parameter derived from Ecoplate data (Choi & Dobbs, 1999, Garland, 1996, Weber & Legge, 2010), was calculated using Equation (2), where \( A_i \) represents the absorbance reading of well \( i \), and \( A_0 \) is the absorbance reading of the blank well.

\[
AWCD = \frac{\sum_{i=1}^{31} (A_i - A_0)}{31} \tag{2}
\]

The Shannon Diversity Index, \( H \), was also calculated from the well data using Equation (3), where \( p_i \) is the ratio of the absorbance of a particular well, \( i \), relative to the sum of absorbances for all 31 wells (Kirk et al., 2005, Weber & Legge, 2010).

\[
H = -\Sigma p_i \ln(p_i) \tag{3}
\]

2.6 | Determination of \( C_{mic} \) and q\( CO_2 \)

\( C_{mic} \) (mg C kg\(^{-1}\)) was determined at 0 days, 1 week, and 40 weeks only, using a procedure based on the chloroform fumigation/K\(_2\)SO\(_4\) extraction method of Jenkinson and Powlson (Jenkinson & Powlson, 1976, Vance, Brookes, & Jenkinson, 1987). \( C_{mic} \) (mg C kg\(^{-1}\)) was calculated from Equation (4), where \( \Delta TOC \) (mg C L\(^{-1}\)) is the difference between the total organic carbon concentrations for the fumigated and nonfumigated samples; \( K_{ec} \) is a soil specific factor for converting extractable carbon to biomass carbon, taken as 0.45 in this case (Sparling, Feltham, Reynolds, West, & Singleton, 1990); \( V \) is the K\(_2\)SO\(_4\) extraction volume (L); and \( m \) is the mass of soil extracted (kg, dry weight). Additionally, the soil metabolic quotient, q\( CO_2 \) (mg CO\(_2\) C g\(^{-1}\) Cmic hr\(^{-1}\)), was calculated from the quotient of the soil respiration rate corrected to mg CO\(_2\) g\(^{-1}\) Cmic hr\(^{-1}\) and \( C_{mic} \).

\[
C_{mic} = \frac{\Delta TOC \times V}{K_{ec} \times m} \tag{4}
\]

2.7 | PCA analysis of dataset

PCA analysis is ideally suited to discern relationships between PAH degradation (16 USEPA PAHs) and trends in the observed biotic parameters (soil respiration rate, AWCD, H, and microbial biomass). Canoco 5 (Smilauer & Leps, 2014) was used to carry out the analysis and visualisation of the PAH degradation data (all 16 PAHs), using the biotic parameters and sole carbon substrate utilisation as explanatory factors.

3 | RESULTS AND DISCUSSION

3.1 | Effect of metal treatment on PAH loss

Figure 1 shows the overall profile for the loss of total extractable PAH concentration from soil microcosms over a 40-week period under different treatment conditions. Up to 80% removal of extractable total PAH concentration was observed in the control microcosm. Corresponding percentage reductions in removal for different metal treatments (20.7% for Pb, 27.7% for Cd, and 37.6% for Hg, at the highest
concentrations) are detailed in Table 2. It is noteworthy from Figure 1 that the curves for the loss of total extractable PAHs are biphasic. In an earlier paper, using data from this same study but focussing only on the kinetics of PAH loss (Deary et al., 2016), we explained this biphasic behaviour by proposing a novel model, whereby the majority of PAH biodegradation takes place on the solid soil phase, but that additional physical processes are responsible for transfer of these compounds to successively more bioinaccessible phases, ultimately to phases that are neither bioaccessible nor chemically extractable. The first step of the biphasic curve comprises both biological and physical processes, whereas the second step comprises only a physical process that ‘locks’ the PAHs within an inaccessible phase of the soil matrix (Deary et al., 2016). It follows that only the first step can be affected by the presence of toxic metals. The relative magnitude of these kinetic processes will vary depending on the soil organic matter (OM) content; the physical processes are likely to be less important for soils with lower OM content than in the present study. Furthermore, the degree to which the loss of individual PAHs can be affected by the presence of metal co-contaminants will be dependent on the relative rate of biodegradation compared with the rate of migration to nonbioaccessible phases of the soil; this will be a function of PAH structure and will be discussed in Section 3.4. The loss of PAHs in the Hg-spiked abiotic control microcosms can be attributed to the biodegradation that occurs during the initial recovery in biological activity (i.e., before respiking at Week 7) and to the migration of PAHs to nonextractable soil phases (Deary et al., 2016). Volatilisation of lower molecular weight PAHs from upper layers of the microcosms is also a possibility, though this was not quantified. Degradation profiles for individual USEPA PAHs under various treatment conditions can be found in Deary et al. (2016), where they were used to develop a kinetic model for biodegradation.

The effect of metal treatment can also be considered for individual PAHs, as detailed in Table 2. The presence of metal co-contaminants has a significant effect on PAH removal, with the greatest reductions (up to 50% for Cd and 41% for Pb) occurring for those PAHs that have the highest number of rings in their structure; these were also the most abundant PAHs at the beginning of the experiment. From Table 2, it is apparent that at the lowest metal-spiked concentrations, Cd has a smaller effect across the range of PAHs than Pb, but that this relationship is reversed at the highest metal-spiked concentrations. It is well known that PAH structure is important in determining the extent of PAH degradation in soil, with the rate generally found to be inversely proportional to the number of rings in the molecule (Cerniglia, 1992, Johnsen et al., 2005, Wammer & Peters, 2005). Other structural considerations, such as whether the molecule is alternant or nonalternant, have also been shown to be important (Wammer & Peters, 2005). A very limited range of bacterial species has been found to grow on PAHs that contain five or more rings, and this has been attributed to limited availability of these compounds in the solution phase, with the consequence that suitable enzymatic pathways for their degradation have had limited opportunity to evolve in soil bacterial communities. Additional metal-induced stress is likely to have a disproportionate effect on the limited microbial communities that are capable of metabolising high molecular weight PAHs.

FIGURE 1 Loss of total extractable polycyclic aromatic hydrocarbons (PAH) concentration in soil microcosms over a period of 280 days in the presence of lead and cadmium at three different concentrations. The curves are the best fit to Equation 14 of Deary et al. (2016) using the following parameters (standard deviations in parentheses): \( k_2; 2.78 (0.24) \times 10^{-2}\ d^{-1}; k_9; 0.27 (0.01) \times 10^{-2}\ d^{-1}; k_{bio2} (\text{Control}); 3.57 (0.21) \times 10^{-2}\ d^{-1}; k_{bio2} (\text{Cd:L}); 2.91 (0.17) \times 10^{-2}\ d^{-1}; k_{bio2} (\text{Cd:M}); 1.72 (0.10) \times 10^{-2}\ d^{-1}; k_{bio2} (\text{Cd:H}); 1.18 (0.07) \times 10^{-2}\ d^{-1}; k_{bio2} (\text{Pb:L}); 2.07 (0.12) \times 10^{-2}\ d^{-1}; k_{bio2} (\text{Pb:M}); 1.80 (0.10) \times 10^{-2}\ d^{-1}; k_{bio2} (\text{Pb: H}); 1.48 (0.09) \times 10^{-2}\ d^{-1}. \) A mercury amended microcosm is included for comparison [Colour figure can be viewed at wileyonlinelibrary.com]

### 3.2 Comparison of relative effects of metal treatment on soil respiration, AWCD, H, and PAH loss

Given the significant effects of metal co-contaminants on PAH loss, as described in the previous section, it is essential to understand how these effects relate to changes in the soil microbial community, as measured by a range of biotic parameters. It is also important to compare the effects of metal co-contaminants on biotic parameters in non-PAH amended soils, as well as the effect that PAH amendment alone has on the soil microbial community.

The effect of PAH and metal treatment on soil respiration rate, Ecotope AWCD, H, and biomass content over a 40-week period is shown in Figure 2a–c, respectively. The corresponding percentage differences relative to the respective controls are shown in Figure 3a–c. Compared with non-PAH-amended microcosms, Figure 2 shows that, at least initially, PAH amendment stimulates both soil respiration and AWCD for most treatments; H is also marginally stimulated. Whilst this difference diminishes with time for the metal-spiked treatments, for the controls it is apparent over the entire duration (apart from week 40, for soil respiration), especially for AWCD. Added PAHs are known to stimulate soil respiration, at least at lower concentrations, serving as a substrate to soil microflora (Lu, Xu, & Chen, 2013), though higher concentrations of specific PAHs are known to have an inhibitory effect (Gogolev & Wilke, 1997).

Figure 2 also shows that for all treatments, including the non-metal-spiked controls, soil respiration and AWCD decline over time. The PAH amended and non-PAH amended control microcosms showed reductions of 79% and 70%, respectively, for soil respiration and 35% and 36%, respectively, for AWCD. The decline in biotic parameters for control treatments could reflect a decrease in substrate...
FIGURE 2  Effect of mercury and different concentrations of lead and cadmium on (a) Respiration rate, (b) Average Well Colour Development (AWCD), and (c) Shannon Diversity Index (H) in soil microcosms. For each treatment, the bars from left to right correspond to 0, 1, 2, 3, 5, 7, 9, 12, 15, 20, 25, 30, 35, and 40 weeks. Orange bars and an asterisk indicate PAH-amended soils; green bars indicate non PAH-amended soils. Treatment labels are defined in Table 1. All results are based on measurements from triplicate microcosms, except soil respiration, which used only one measurement. The error bars indicate ±1σ [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 3  Data from Figure 2 shown as relative percentage differences compared with the control sample. For each treatment, the bars from left to right correspond to 0, 1, 2, 3, 5, 7, 9, 12, 15, 20, 25, 30, 35, and 40 weeks. Orange bars and an asterisk indicate PAH-amended soils; green bars indicate non-PAH-amended soils. Treatment labels are defined in Table 1 [Colour figure can be viewed at wileyonlinelibrary.com]
quality with time; there is no replenishment of nutrients, as there would be in a natural environment (Stefanowicz, Niklińska, & Laskowski, 2009; Vanhala, 2002). It could also reflect the adverse effects of experimental soil handling processes, which are known to influence the viability of those microbial species that are most sensitive to environmental conditions (Northcott & Jones, 2000, Reid, Northcott, Jones, & Semple, 1998). These results contrast with those for Hg in Figure 2c, which showed that, with the exception of Hg treatments, for non-PAH-amended microcosms, there is only a minimal decrease in this parameter during the experiment. There is, however, a more defined decrease for PAH-amended soils.

The Hg treatments showed a sizeable initial reduction in H, followed by a significant recovery, before a further reduction after the second spiking that was necessary to maintain abiotic conditions. It is noteworthy that in both the absence and presence of PAHs, the reduction in H, relative to the control, never reaches the same level as it did with the initial Hg treatment, implying the emergence of some level of community tolerance. Niklinska, Chodak, and Laskowski (2006) have demonstrated the development of significant community tolerance of the soil microbial community to Hg in just 1 week, and Muniz, Lacarta, Pata, Jimenez, and Navarro (2014) have shown similar trends to ours for H over a period of only 100 hr in Cd amended soils.

For the majority of treatments, the presence of metal contaminants inhibits the rate of soil respiration compared with the control samples (Figure 3a); however, for the lowest Pb and Cd concentrations, both in the presence and absence of PAHs, there appears to be some stimulation of respiration at certain periods during the study. The relative decrease in AWCD for the majority of treatments shown in Figure 3b is greatest for the PAH-amended microcosms, though for soil respiration rate and H, Figures 3a,c, respectively, there is a less discernable difference. Literature studies have shown that the combined action of PAHs and metal co-contaminants is greater than the effect of either on their own, for example, Cd/pyrene (Lu et al., 2013) and Cd/phenanthrene (Shen, Cao, Lu, & Hong, 2005). It is also the case that in the absence of PAHs, Cd has a more significant inhibitory effect on AWCD, yet the reverse is true when PAHs are also present.

3.3 | \(C_{\text{mic}}\) and qCO2

\(C_{\text{mic}}\) values shown in Figure 4a are consistent with the decreases observed for AWCD and soil respiration in Figures 2 and 3. This measurement was carried out at only 3 points during the experiment (premetal spiking, after 1 week, and at 40 weeks). The results showed that \(C_{\text{mic}}\) values (a) are stimulated in the presence of PAHs; (b) decline in all samples over the duration of the experiment; (c) decline at a greater rate for higher metal concentrations; (d) are more inhibited by Pb than Cd, and (e) exhibited the greatest reductions in the presence of PAHs, notwithstanding the initial stimulation. The relative toxicities of Pb compared with Cd may be dependent on soil OM concentration; in a study on a low organic carbon soil (1.93%), a spiking level of 100 mg kg\(^{-1}\) Cd showed greater inhibitory effects on \(C_{\text{mic}}\) than that of a 1,000 mg kg\(^{-1}\) spiking of Pb (Akmal, Xu, Li, Wang, & Yao, 2005). There are, however, wide disparities in the literature for relative toxicity of metals towards soil microflora (Baath, 1989; Giller, Witter, & Mcgrath, 1998).

qCO2, Figure 4b, is a parameter considered to be inversely related to the efficiency with which the soil microflora can metabolise soil OM (Anderson & Domsch, 1990, Lu et al., 2013). An increase in qCO2 is often interpreted as an indication of stress on microbial communities, for example, as a response to contamination or disturbance (Brookes & Mcgrath, 1984, Fließbach, Martens, & Reber, 1994, Wardle & Ghani, 1995). Whilst this has been a matter of debate, Wardle and Ghani in their critique of its use, conclude that it is ‘most appropriately used as an index of adversity of environmental conditions’ (Wardle & Ghani, 1995). In such conditions, the soil microflora requires increased energy expenditure for physiological adaptations necessary for survival, with less energy available for incorporation into biomass (Killham, 1985; Schindlbacher et al., 2011; Vittori Antisari, Carbone, Gatti, Vianello, & Nannipieri, 2013; Zhang et al., 2010). qCO2 has been shown in the literature to be a sensitive marker for metal contamination, both for long-term polluted sites (Renella, Mench, Landi, & Nannipieri, 2005; Yao, Xu, & Huang, 2003; Zhang et al., 2010) and in microcosm experiments (dos Santos et al., 2012, Lu et al., 2013, Vittori Antisari et al., 2013).

Our results for qCO2 showed that PAH-amended soils display a metal concentration-related increase in qCO2 with Pb exerting a greater effect than Cd. For almost all treatments, there is a decrease in qCO2 at 40 weeks compared with 1 week; similar trends have been observed in the literature (Vittori Antisari et al., 2013) and may reflect the emergence of some community tolerance. In the absence of PAHs, apart from the Hg treatment, the trend is less clear, with only small effects (positive and negative) on qCO2, though with Pb having a

![FIGURE 4](https://example.com/figure4.png)
marginally higher effect and also showing a decrease in qCO₂ at 40 weeks compared with 1 week. Hg-spiked soils showed the highest qCO₂ values, particularly for the PAH-amended treatment. Muller, Westergaard, Christensen, and Sorensen (2001) reported significant decreases in bacterial biomass (though not fungal) in long-term Hg-contaminated sites (approximately 500 mg kg⁻¹ Hg).

### 3.4 PCA analysis

PCA analysis was used to elucidate the relationships between biotic parameters and the extent of degradation of the PAHs. Figure 5 shows a time series (1, 5, 12, 20, 30, and 40 weeks) of PCA analyses carried out on PAH degradation (orange circles) under the different...
treatment conditions, in which the size of the circle corresponds to the relative (within each week) median percentage loss of the 16 USEPA PAHs that were analysed (this analysis is a modified version of the Functional Trait Diversity Diagrams of Smilauer and Leps (2014). As an aid to interpreting the separation of the treatments along the two ordination axes, two sets of arrows, corresponding to explanatory variables, are included. The red arrows correspond to measured biotic parameters (soil respiration, AWCD, H, and biomass, where available), and the grey arrows show the influence of individual sole carbon substrate utilisation. In addition, the centroids for degradation of individual PAHs across all treatments are shown and grouped according to the number of rings present: (a) dark blue for two and three-ring PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, and anthracene); (b) light blue for four-ring PAHs (fluoranthene, pyrene, benzo[a]anthracene, and chrysene); and (c) green for five and six-ring PAHs (benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[123-cd]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene). The percentage variance explained by each ordination axis is shown in the bottom left-hand corner of the plots. For each of the weeks, the greatest proportion of the variance is explained by the first ordination axis, with the percentage degradation increasing from right to left. Statistical parameters for the PCA plots are summarised in Table 3.

Looking at the trends in explanatory factors across the weeks in Figure 5, we see at Week 1 that the extent of degradation is largely correlated with soil respiration rate, AWCD, and biomass content. In contrast, H, relating to the diversity of substrate utilisation, and thus to the diversity of the microflora, is negatively correlated with the extent of PAH degradation and with the other biotic parameters. Similar negative relationships between microbial diversity and extent of biodegradation have been observed in the literature for diesel contamination, where it has been attributed to reduced competitive stress on the key biodegrading species, as noted in the introduction (Bell et al., 2013; Jung et al., 2016). After Week 12, H becomes positively correlated with the other biotic parameters and thus with increasing overall degradation.

Figure 5 also allows us to discern the trends in degradation of PAHs according to structure. At Week 1, there is an overlap of the three structural groups, indicating that, except for a couple of outliers in the 5- to 6-ring group, the treatment type has minimal structure-related impact on the extent of degradation. However, for the subsequent weeks, there is a clear separation of the 5- to 6-ring group from the other two groups along the first PCA axis, which largely correlates with higher values of the biotic parameters. The 2- to 3-ring and 4-ring PAH groups remain overlapped in all cases, though the latter is consistently displaced to the left of the 2 to 3-ring group in panels (b) to (f). These separations show that after Week 1, the higher metal concentration treatments and their impact on biotic parameters are having a differential effect on PAH degradation depending on the number of rings present in the molecule. Degradation of 5- to 6-ring PAHs is associated with higher values of AWCD, soil respiration, and microbial diversity (after Week 12 for the latter), indicating that more metabolically active and diverse microbial communities are required to metabolise these PAHs.

Finally, associations can be made between the extent of utilisation of sole carbon sources (well colour development) and the parameters discussed above. Across the 40 weeks of the study, those substrates that are most consistently correlated with AWCD, soil respiration, and extent of biodegradation are A4 (L-arginine), B1 (pyruvic acid methyl ester), B3 (D-galacturonic acid), D2 (D-mannitol), D3 (4-hydroxy benzoic acid), E2 (N-acetyl-D-glucosamine), F2 (D-}

### Table 3: Statistics for the Principal Component Analysis shown in Figure 5

| Time   | Statistic                        | Axis 1       | Axis 2       | Axis 3       | Axis 4       |
|--------|----------------------------------|--------------|--------------|--------------|--------------|
| Week 1 | Eigenvectors                      | 0.5275       | 0.1922       | 0.1283       | 0.0961       |
|        | Explained variation (cumulative)a | 49.4         | 72.37        | 84.17        | 93.29        |
|        | Pseudo-canonical correlation (suppl.)b | 0.955       | 0.8772       | 0.8604       | 0.7819       |
| Week 5 | Eigenvectors                      | 0.7272       | 0.1826       | 0.0365       | 0.0280       |
|        | Explained variation (cumulative)  | 65.87        | 86.74        | 91.9         | 96.29        |
|        | Pseudo-canonical correlation (suppl.) | 0.8387      | 0.7007       | 0.567        | 0.6299       |
| Week 12| Eigenvectors                      | 0.7535       | 0.1198       | 0.0550       | 0.0395       |
|        | Explained variation (cumulative)  | 61.26        | 79.00        | 88.42        | 95.07        |
|        | Pseudo-canonical correlation (suppl.) | 0.9206      | 0.9804       | 0.3352       | 0.2502       |
| Week 20| Eigenvectors                      | 0.8372       | 0.0655       | 0.0500       | 0.0323       |
|        | Explained variation (cumulative)  | 72.3         | 83.32        | 91.92        | 97.65        |
|        | Pseudo-canonical correlation (suppl.) | 0.8423      | 0.759        | 0.8996       | 0.5965       |
| Week 30| Eigenvectors                      | 0.8818       | 0.0507       | 0.0350       | 0.0181       |
|        | Explained variation (cumulative)  | 80.37        | 89.00        | 94.68        | 97.87        |
|        | Pseudo-canonical correlation (suppl.) | 0.9548      | 0.5809       | 0.6974       | 0.4897       |
| Week 40| Eigenvectors                      | 0.8966       | 0.0551       | 0.0281       | 0.0117       |
|        | Explained variation (cumulative)  | 80.18        | 90.97        | 96.56        | 98.48        |
|        | Pseudo-canonical correlation (suppl.) | 0.9277      | 0.517        | 0.7773       | 0.4027       |

*aCumulative percentage of variance in the response data explained by the axes.

*bMultiple correlation coefficient R of the axis with the supplementary variables (H, AWCD, and soil respiration).
glucosamine acid), and F3 (itaconic Acid), while those substrates showing a negative correlation with these parameters are C1 (Tween 40), C4 (L-phenylalanine), D1 (Tween 80), E1 (α-cyclodextrin), E3 (γ-hydroxybutyric Acid), E4 (L-threonine), H1 (α-D-lactose), H2 (D,L-α-glycerol phosphate), and H3 (D-malic acid).

4 | CONCLUSIONS

The results described in this paper represent a substantial study of the biodegradation of 16 US EPA PAHs over a 40-week period, adding to the limited body of work involving PAH removal in long-term studies, and in particular, in soils of high OM. The additional consideration of the effects of metal treatments on biodegradation and the relationship between PAH removal and various measures of microbial community function gives a useful insight into the processes occurring in sites that are cocontaminated with metals and PAHs. The study has also looked at the effect of metal contamination on soil microbial function in the absence of PAHs.

Whilst Pb, Cd, and Hg co-contaminants do have a substantial effect on overall and individual PAH removal efficiencies, the simultaneously determined biotic parameters show a resilient soil microbial community that is capable of significant biodegradation even in soils that are highly contaminated with metals. Also, it is likely that significant PAH removal may occur via a physical process, whereby PAHs successively migrate to soil phases are neither bioavailable nor extractable. Finally, soil microbial diversity was observed to be negatively associated with greater overall PAH removal for the initial period of the experiment (up until Week 12). Negative associations between microbial diversity and biodegradation have been reported in the literature for other organic contaminants, but as far as we are aware, this is the first such report for PAHs.

ACKNOWLEDGMENT

We are grateful to Northumbria University for financially supporting this study.

ORCID

Michael E. Deary http://orcid.org/0000-0002-2370-1243

REFERENCES

Akmal, M., Xu, J. M., Li, Z. J., Wang, H. Z., & Yao, H. Y. (2005). Effects of lead and cadmium nitrate on biomass and substrate utilization pattern of soil microbial communities, Chemosphere, 60, 508–514. https://doi.org/10.1016/j.chemosphere.2005.01.001

Anderson, T. H., & Domsch, K. H. (1990). Application of ecophysiological quotients (QCo2 and Qd) on microbial biomass from soils of different cropping histories. Soil Biology & Biochemistry, 22, 251–255. https://doi.org/10.1016/0038-0717(90)90094-G

Atagana, H. I. (2006). Biodegradation of polycyclic aromatic hydrocarbons in contaminated soil by biostimulation and bioaugmentation in the presence of copper(II) ions. World Journal of Microbiology & Biotechnology, 22, 1145–1153. https://doi.org/10.1007/s11274-006-9155-z

Baath, E. (1989). Effects of heavy metals in soil on microbial processes and populations (a review). Water Air and Soil Pollution, 47, 335–379. https://doi.org/10.1007/BF00279331

Baboshin, M. A., & Golovleva, L. A. (2012). Aerobic bacterial degradation of polycyclic aromatic hydrocarbons (PAHs) and its kinetic aspects.

Microbiology, 81, 639–650. https://doi.org/10.1134/S0026261712060021

Baird, W. M., Hooven, L. A., & Mahadevan, B. (2005). Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. Environmental and Molecular Mutagenesis, 45, 106–114. https://doi.org/10.1002/em.20095

Bell, T. H., Yergeau, E., Juck, D. F., Whyte, L. G., & Greer, C. W. (2013). Alteration of microbial community structure affects diesel biodegradation in an Arctic soil. Fems Microbiology Ecology, 85, 51–61. https://doi.org/10.1111/1574-6941.12102

Bezza, F. A., & Chirwa, E. M. N. (2017). The role of lipopeptide biosurfactant on microbial remediation of aged polycyclic aromatic hydrocarbons (PAHs)-contaminated soil. Chemical Engineering Journal, 309, 563–576. https://doi.org/10.1016/j.cej.2016.10.055

Brookes, P. C., & Mcgrath, S. P. (1984). Effect of metal toxicity on the size of the soil microbial biomass. Journal of Soil Science, 35, 341–346. https://doi.org/10.1111/j.1365-2389.1984.tb00288.x

Cerniglia, C. E. (1992). Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation, 3, 351–368. https://doi.org/10.1007/978-94-011-1672-5_16

Cerniglia, C. E. (1997). Fungal metabolism of polycyclic aromatic hydrocarbons: Past, present and future applications in bioremediation. Industrial Microbiology and Technology, 19, 324–333. https://doi.org/10.1038/sj.jim.29004

Chen, J., Li, S., Xu, B., Su, C., Jiang, Q., Zhou, C., … Xiao, M. (2017). Characterization of Burkholderia sp. XTB-5 for phenol degradation and plant growth promotion and its application in bioremediation of contaminated soil. Land Degradation & Development, 28, 1091–1099. https://doi.org/10.1002/ldr.2646

Choi, K. H., & Dobbs, F. C. (1999). Comparison of two kinds of Biolog microplates (GN and ECO) in their ability to distinguish among aquatic microbial communities. Journal of Microbiological Methods, 36, 203–213. https://doi.org/10.1016/S0167-7012(99)00034-2

Deary, M. E., Ekumankama, C. C., & Cummings, S. P. (2016). Development of a novel kinetic model for the analysis of PAH biodegradation in the presence of lead and cadmium co-contaminants. Journal of Hazardous Materials, 307, 240–252. https://doi.org/10.1016/j.jhazmat.2015.12.015

Derry, A. M., Staddon, W. J., & Trevers, J. T. (1998). Functional diversity and community structure of microorganisms in uncontaminated and creosote-contaminated soils as determined by sole carbon-source-utilization. World Journal of Microbiology & Biotechnology, 14, 571–578. https://doi.org/10.1023/A:1008812821516

dos Santos, E. D., Silva, I. S., Simoes, T. H. N., Simioni, K. C. M., Oliveira, V. M., Grossman, M. J., & Durrant, L. R. (2012). Correlation of soil microbial community responses to contamination with crude oil with and without chromium and copper. International Biodeterioration & Biodegradation, 70, 104–110. https://doi.org/10.1016/j.ibiod.2012.01.010

European Commission. (2012). The implementation of the soil thematic strategy and ongoing activities (COM(2012) 46 final). Brussels: European Commission. https://doi.org/10.4161/intv.21223

Fließbach, A., Martens, R., & Reber, H. H. (1994). Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. Soil Biology & Biochemistry, 26, 1201–1205. https://doi.org/10.1016/0038-0717(94)90144-9

Gan, S., Lau, E. V., & Ng, H. K. (2009). Remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). Journal of Hazardous Materials, 172, 532–549. https://doi.org/10.1016/j.jhazmat.2009.07.118

Garland, J. L. (1996). Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biology & Biochemistry, 28, 213–221. https://doi.org/10.1016/0038-0717(95)00112-3

Giller, K. E., Witter, E., & Mcgrath, S. P. (1998). Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: A review.
Stefanowicz, M. A., Niklifirska, M., & Laskowski, R. (2009). Pollution-induced tolerance of soil bacterial communities in meadow and forest ecosystems polluted with heavy metals. European Journal of Soil Biology, 45, 363–369. https://doi.org/10.1016/j.ejsobi.2009.05.005

Straube, W. L., Nestler, C. C., Hansen, L. D., Ringleberg, D., Pritchard, P. H., & Jones-Meehan, J. (2003). Remediation of polycyclic aromatic hydrocarbons (PAHs) through landfarming with biostimulation and bioaugmentation. Acta Biotechnologica, 23, 179–196. https://doi.org/10.1002/abio.200390025

Sun, M., Luo, Y., Teng, Y., Jia, Z., Li, Z., & Deng, S. (2013). Remediation of polycyclic aromatic hydrocarbon and metal-contaminated soil by successive methyl-β-cyclodextrin-enhanced soil washing-microbial augmentation: A laboratory evaluation. Environmental Science and Pollution Research, 20, 976–986. https://doi.org/10.1007/s11356-012-1064-0

Sun, T.-R., Cang, L., Wang, Q.-Y., Zhou, D.-M., Cheng, J.-M., & Xu, H. (2010). Roles of abiotic losses, microbes, plant roots, and root exudates on phytoremediation of PAHs in a barren soil. Journal of Hazardous Materials, 176, 919–925. https://doi.org/10.1016/j.jhazmat.2009.11.124

Thavamani, P., Malik, S., Beer, M., Meghraj, M., & Naidu, R. (2012). Microbial activity and diversity in long-term mixed contaminated soils with respect to polycyclic aromatic hydrocarbons and heavy metals. Journal of Environmental Management, 99, 10–17. https://doi.org/10.1016/j.jenvman.2011.12.030

The International Organization for Standardization (2002). ISO 16072: Soil quality laboratory methods for determination of microbial soil respiration. London: The British Standards Institution.

Tyagi, M., Da Fonseca, M. M. R., & De Carvalho, C. C. C. R. (2011). Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. Biodegradation, 22, 231–241. https://doi.org/10.1007/s10532-010-9394-4

Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry, 19, 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Vanhala, P. (2002). Seasonal variation in the soil respiration rate in coniferous forest soils. Soil Biology & Biochemistry, 34, 1375–1379. https://doi.org/10.1016/S0038-0717(02)00061-5

Vittori Antisari, L., Carbone, S., Gatti, A., Vianello, G., & Nannipieri, P. (2013). Toxicity of metal oxide (CeO2, Fe3O4, SnO2) engineered nanoparticles on soil microbial biomass and their distribution in soil. Soil Biology & Biochemistry, 60, 87–94. https://doi.org/10.1016/j.soilbio.2013.01.016

Wammer, K. H., & Peters, C. A. (2005). Polycyclic aromatic hydrocarbon biodegradation rates: A structure based study. Environmental Science & Technology, 39, 2571–2578. https://doi.org/10.1021/es048939y

Wardle, D. A., & Ghani, A. (1995). A critique of the microbial metabolic quotient (qCO2) as a bioindicator of disturbance and ecosystem development. Soil Biology & Biochemistry, 27, 1601–1610. https://doi.org/10.1016/0038-0717(95)00093-T

Weber, K. P., & Legge, R. L. (2010). Community level physiological profile. In S. P. Cummings (Ed.), Bioremediation: methods and protocols. New York: Humana Press.

Yao, H., Xu, J. H., & Huang, C. (2003). Substrate utilization pattern, biomass and activity of microbial communities in a sequence of heavy metal-polluted paddy soils. Geoderma, 115, 139–148. https://doi.org/10.1016/s0016-7061(03)00083-1

Zhang, F., Li, C., Tong, L., Yue, L., Li, P., Ciren, Y., & Cao, C. (2010). Response of microbial characteristics to heavy metal pollution of mining soils in central Tibet, China. Applied Soil Ecology, 45, 144–151. https://doi.org/10.1016/j.apsoil.2010.03.006

SUPPORTING INFORMATION
Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Deary ME, Ekumankama CC, Cummings SP. Effect of lead, cadmium, and mercury co-contaminants on biodegradation in PAH-polluted soils. Land Degrad Dev. 2018;29:1583–1594. https://doi.org/10.1002/ldr.2958