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The Effect of Rhizosphere Soil and Root Tissues Amendment on Microbial Mineralisation of Target ^{14}C–Hydrocarbons in Contaminated Soil

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Abstract The effect of rhizosphere soil or root tissues amendments on the microbial mineralisation of hydrocarbons in soil slurry by the indigenous microbial communities has been investigated. In this study, rhizosphere soil and root tissues of reed canary grass (*Phalaris arundinacea*), channel grass (*Vallisneria spiralis*), blackberry (*Rubus fructicosus*) and goat willow (*Salix caprea*) were collected from the former Shell and Imperial Industries (ICI) Refinery site in Lancaster, UK. The rates and extents of ^{14}C–hydrocarbons (naphthalene, phenanthrene, hexadecane or octacosane) mineralisation in artificially spiked soils were monitored in the absence and presence of 5% (wet weight) of rhizosphere soil or root tissues. Respirometric and microbial assays were monitored in fresh (0 d) and pre–incubated (28 d) artificially spiked soils following amendment with rhizosphere soil or root tissues. There were significant increases ($P < 0.001$) in the extents of ^{14}C–naphthalene and ^{14}C–phenanthrene mineralisation in fresh artificially spiked soils amended with rhizosphere soil and root tissues compared to those measured in unamended soils. However, amendment of fresh artificially spiked soils with rhizosphere soil and root tissues did not enhance the microbial mineralisation of ^{14}C–hexadecane or ^{14}C–octacosane by indigenous microbial communities. Apart from artificially spiked soil systems containing naphthalene (amended with reed canary grass and channel grass rhizosphere) and hexadecane amended with goat willow rhizosphere, microbial mineralisation of hydrocarbons was further enhanced following 28 d soil–organic contaminants pre–exposure and subsequent amendment with rhizosphere soil or root tissues. This study suggests that organic chemicals in roots and/or rhizosphere can enhance the microbial degradation of petroleum hydrocarbons in freshly contaminated soil by supporting higher numbers of hydrocarbon–degrading populations, promoting microbial activity and/or enhancing bioavailability of organic contaminants.

Keywords: *microbial mineralization, rhizosphere soil, root tissue, hydrocarbons, PAHs*

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1. Introduction

Petroleum hydrocarbon contamination of soil and marine environment is one of the major environmental problems associated with petroleum resources exploration and development across the world [1]. Of the various petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants of serious concern because they persist in nature and some PAHs have been identified as carcinogens, mutagens, or teratogens. PAHs, which are relatively stable contaminants and recalcitrant in soils, have multiplicity of sources that can be broadly classified as diagenetic, pyrogenic, or petrogenic in origin [2,3]. Evidence from several studies has shown that risks of adverse health effects associated with contaminated soils relates to the likelihood and magnitude of human exposure (direct or indirect) to toxic substances in soil [4,5,6]. However, microbial transformation and growth–linked mineralisation by indigenous microbial communities play a major role in degradation of most petroleum–derived contaminants, such as benzene, toluene, ethylbenzene and xylenes (BTEX), aliphatic and polycyclic aromatic hydrocarbons [7,8]. Compare to the expensive engineering techniques, biological remediation (bioremediation) techniques, such as phytoremediation and rhizoremediation, are cost effectiveness as well as environmental–friendly approach for remediation for petroleum–contaminated soils.

Phytoremediation involves the use of plants and their associated microbes (in a symbiotic interaction) to remove, transform and/or degrade inorganic and organic contaminants in soil, sediments and groundwater [9,10,11]. Phytoremediation strategies for organic contaminants such as petroleum hydrocarbons can be grouped into direct

phytoremediation (*in planta*) and phytoremediation *ex planta* [12,13,14]. The latter is based on a synergistic relationship between root exudates (substances that are released into the rhizosphere) and metabolic activities of rhizosphere–associated microbes [15]. Plant–microbe–soil interactions have played a vital role in reshaping our contaminated environment through a series of biological, physical–chemical processes and metabolic transformations. Plants employ several mechanisms to remediate soils contaminated with petroleum hydrocarbons [8,16] and plant–enhanced microbial degradation of organic contaminants in soil have been documented in several studies [12,15,17,18,19,20,21]. Evidence for the potential role of plants in hydrocarbon degradation through the rhizosphere effect have been provided; wherein plants exude organic compounds through their roots, influencing the abundance, diversity, or activity of potential rhizospheric hydrocarbon–degrading microbes [12]. Root exudates have traditionally been grouped into (i) low molecular weight compounds (LMWCs: simple sugar, amino acids, fatty acids, organic acids, phenolic compounds, aliphatic and/or aromatic compounds), and (ii) high molecular weight compounds (HMWCs: polysaccharides, polygalatic acids and proteins) [22,23,24]. Root exudates can be utilized by some soil microbial communities as growth substrates [25], and can act as co-metabolites for the degradation of persistent organic pollutants [26,27,28,29]. It has been found that the respiration of rhizosphere soil is greater than that of the bulk soil, since CO_2 can originate not only from microbial respiration of soil organic C, but also from root respiration and microbial decomposition of rhizodeposition [30].

Root exudates potentially supply microbes with micronutrients and the exudation of organic compounds from roots is an important process in mediating plant–microbe interactions. The emission and utilization of volatile organic compounds (VOCs) within the rhizosphere form a significant part of the carbon cycle [31, 32]. The emission of VOCs from soil, either by roots or by decomposing biomass may enhance the biodegradation of organic contaminants [33,34,35]. Few studies have demonstrated that, depending on the (bio)available concentration and solubility in soil, plant–derived chemicals such as biogenic VOCs [36], hydroxycinnamic [16] and flavonoids [8] can either stimulate or inhibit microbial mineralisation of organic contaminants. Aliphatic and aromatic acids occur naturally in plant roots and whether or not these compounds are present in the rhizosphere in quantities sufficient to stimulate microbial mineralisation of organic contaminants in soil is not fully understood. Although phytoremediation has been extensively investigated, there is paucity of information on specific mechanisms and the complex role of plant secreted chemical compounds on microbial degradation of organic contaminants in soil.

This study investigated the influence of rhizosphere soil or root tissues of reed canary grass (*Phalaris arundinacea*), channel grass (*Vallisneria spiralis*), blackberry (*Rubus fructicosus*) and goat willow (*Salix caprea*) amendments on microbial mineralisation of target ^{14}C–hydrocarbons (naphthalene, phenanthrene, hexadecane or octacosane) in fresh (0 d) and pre–incubated (28 d) artificially spiked soils.

2. Materials and Methods

2.1. Materials

The chemicals, naphthalene (>96%); [UL–^{14}C] naphthalene (specific activity = 2–10 mCi mmol^{-1}, radiochemical purity > 95%), phenanthrene (>96%); [9–^{14}C] phenanthrene (specific activity = 50 mCi mmol^{-1}, radiochemical purity 99.6%), *n*–hexadecane (>99%); [1–^{14}C] *n*–hexadecane (specific activity = 7.5 mCi mmol^{-1}, radiochemical purity 98.6%), octacosane (≤99%), [14, 15–^{14}C] octacosane (activity = 7.5 mCi mmol^{-1}, radiochemical purity 98%) were acquired from Sigma–Aldrich, UK and American Radiolabeled Chemicals (ARC) UK. Fisher Scientific UK supplied the nutrient agar, sodium hydroxide (NaOH) used for the CO_2 traps and the mineral basal salts (MBS) solution reagents. Ringer's solution pellets and plate count agar (PCA) powder were obtained from Oxoid Ltd, UK. Schott UK Ltd supplied the 250 ml Schott Duran® bottles with Teflon™ lined screw caps and 500 ml Schott Duran® amber jars (wide mouth) with Teflon–lined™ screw caps. The metal fittings used to make the respirometers were obtained from RS Components Ltd, UK. The 7 ml glass scintillation vials and Goldstar liquid scintillation cocktail were supplied by Meridian UK.

2.2. Sampling, Sample Preparation and Characterisation

A pristine agricultural soil (Dystric Cambisol) was collected from the A horizon (5 – 20 cm depth) from Myerscough Agricultural College, Lancashire, UK. In addition, plants with attached roots and root–associated rhizosphere soil used in this study were collected from the former Shell and Imperial Chemical Industries (ICI) Petroleum Refinery site at Middleton Woods (Lancaster, UK) which has been derelict since 1977 and has developed a range of wildlife habitats since it was decommissioned. The plants sampled included reed canary grass (*Phalaris arundinacea*), channel grass (*Vallisneria spiralis*), blackberry (*Rubus fructicosus*) and goat willow (*Salix caprea*). Prior to spiking, the pasture soil was air–dried for 24 h and subsequently homogenised by passing through a 2 mm mesh sieve to remove stones and residual plant materials [16]. The field moisture content was determined in triplicate by oven drying at 105 °C for 24 h [37]. The soil that was removed from around the roots was used as rhizosphere soil and the entire root system was chopped into 0.2 cm pieces and macerated in a blender prior to the experiment. The homogenised soil, rhizosphere soil and plant root samples were stored in the dark at 4°C prior to the experimental setup.

The physicochemical properties of the pasture soil was determined using standard techniques. Soil texture was determined using sedimentation technique and the soil pH (ratio 10 g soil:25 ml dH_2O) was determined using a calibrated pH meter (Model 657R-00). The total extractable organic carbon content was determined using loss on ignition (LOI) method (450°C for 24 h) and a Carlo Erba CHNS–OEA 1108 CN–Elemental analyzer was used to determine the total carbon and nitrogen

contents [8,16]. In addition, the phosphate content was determined by acid digestion with HNO_3 and a phosphate reducing agent (neutralized with NaOH) was used to develop the characteristic blue colour for spectrometric determination at 882 nm (Cecil CE 1011 UV Spectrometer). In this study, best laboratory practices were adopted to ensure quality control and all measurements were triplicate determinations.

2.3. Soil Spiking with Target Hydrocarbons

The air–dried homogenised pasture soil samples were rehydrated with de–ionised water to 35% by weight (original field moisture content) prior to spiking. Samples of the hydrated soil (350 g wet weight) were then spiked with ^{12}C–labelled naphthalene, phenanthrene, hexadecane or octacosane using acetone as the carrier solvent to give a final ^{12}C–hydrocarbon concentration of 10 mg kg^{-1} (dry weight). The carrier solvent in the artificially spiked soil samples was allowed to vent for 2 hours under a fumehood to minimize the impact on the indigenous soil microbial communities. Homogeneity of the spiked soil samples was achieved by blending, wherein soils were manually mixed in glass bowls using a stainless steel spoon [38]. Furthermore, control samples (analytical blanks) consisting of rehydrated soil (350 g wet weight) only and the artificially spiked soil samples were stored in 500ml Schott Duran® amber glass jars in triplicates with loosely fitted Teflon–lined™ screw caps to ensure ambient oxygen exchange [8,16]. All samples of artificially spiked soil and controls stored in amber jars were incubated in darkness at 21.5 ± 0.5°C and 45% relative humidity. The artificially spiked soils and controls were sampled at 0 d and after 28 d pre–incubation for both mineralisation assays and microbial analysis.

2.4. Mineralisation of ^{14}C–Hydrocarbons in Soil

The rates and extents of microbial mineralisation of ^{14}C–hydrocarbons (naphthalene, phenanthrene, hexadecane and octacosane) in the artificially spiked soil samples were determined using respirometric techniques following the procedure developed by Reid et al. [39]. Respirometers were set up in triplicates using modified 250 ml Schott Duran® bottles containing 10 g ± 0.1 g soil (wet weight) and 30 ml autoclaved minimal basal salt (MBS) solution [38, 40]. Each of the respirometers was spiked with target hydrocarbon standards prepared in toluene to deliver ^{12}C–hydrocarbons (naphthalene, phenanthrene, hexadecane or octacosane) concentration of 10 mg kg^{-1} soil dry weight with an associated ^{14}C–activity of 83 Bq g^{-1} soil dry weight. In each of the respirometer, a 7 ml scintillation vial containing 1 ml NaOH (1 M) solution was suspended from the lid to trap $^{14}CO_2$ that evolved as a result of microbial mineralisation of target ^{14}C–hydrocarbons. In addition, unamended respirometers were prepared as outlined above, with 10 ± 0.1 g naphthalene, phenanthrene, hexadecane or octacosane spiked soil samples (wet weight) and 30 ml of autoclaved MBS solution.

To evaluate phyto-/rhizo-remediation strategies to promote the mineralisation of target hydrocarbons by indigenous soil microbial communities, respirometers were also prepared as described above with the following treatments: (i) fresh (0 d) artificially spiked soils amended with 5% (wet weight) of rhizosphere soil and root tissues of reed canary grass (*Phalaris arundinacea*), channel grass (*Vallisneria spiralis*), blackberry (*Rubus fructicosus*) and goat willow (*Salix caprea*), (ii) pre-incubated (28 d) artificially spiked soils amended with 5% (wet weight) of rhizosphere soil and root tissues of reed canary grass (*Phalaris arundinacea*), channel grass (*Vallisneria spiralis*), blackberry (*Rubus fructicosus*) and goat willow (*Salix caprea*) prior to mineralisation assays, and (iii) artificially spiked soil samples with no amendment were used as controls to assess any increase in rates and extents of microbial mineralisation of the target ^{14}C–hydrocarbons.

In order to ensure analytical quality control, respirometers containing only 10 ± 0.1 g rehydrated soil (wet weight) and 30 ml of autoclaved MBS solution were also prepared. During the mineralisation assay, the sealed respirometers were placed securely on a bench–top orbital shaker (Janke and Kunkel, IKA® – Labortechnik KS 250) at 21 °C and shaken at 100 rpm to agitate and ensure adequate mixing of the soil slurry [8, 16]. The spent $^{14}CO_2$ traps were replaced every 24 h over 14 d sampling period and 5 ml of Goldstar scintillation fluid added to each spent $^{14}CO_2$ trap. Prior to counting of the level of ^{14}C–activity, the spent $^{14}CO_2$ trap samples were stored in darkness > 12 h to reduce the effects of chemilumiescence [16] and subsequently quantified using a Packard Canberra Tri-Carb 2300TR liquid scintillation counter [41]. The lag phases, rates and extents of target ^{14}C–hydrocarbons mineralisation in the soil slurries were calculated based on the percentage of trapped $^{14}CO_2$ over the total pool of ^{14}C–labelled carbon.

2.5. Enumeration of Total Heterotrophic and Hydrocarbon–Degrading Bacteria

The number of total heterotrophic bacteria (THB) and indigenous naphthalene, phenanthrene, hexadecane and octacosane–degrading bacteria were evaluated following standard aseptic plate count techniques [42]. In brief, 1.0 ± 0.1 g soil samples were extracted with 10 ml of ¼ strength sterile Ringer's solution following proper mixing in sterile sample bottles. Aliquots (0.1 ml) of each sample were subsequently serially diluted (up to 10^{-7}) with Ringer's solution. Starting with the highest dilution, 0.1 ml of each dilution was inoculated onto plate count agar for THB and agar–agar plates amended with 0.1% naphthalene, phenanthrene, *n*–hexadecane or *n*–octacosane as the sole carbon source for naphthalene, phenanthrene, hexadecane and octacosane–degrading bacteria. After drying at room temperature, the inoculated plates are stacked upside down in piles and incubated at 25 ± 0.5 °C in darkness. The cell numbers of THB were counted after 48 h and > 5 d for naphthalene, phenanthrene, hexadecane and octacosane–degrading bacteria. The total number of microbial cells were expressed as colony–forming units per gram of dry soil (CFU g^{-1}).

2.6. Statistical Analysis of Data

The results were statistically analysed at various time intervals after blank correction and statistically verified using *t*–tests after normality and equal variance tests (Tukey test, $P \leq 0.05$) using statistical software – SigmaStat®, Version 3.5 (Systat Software Inc., USA). The

microbial mineralisation profiles of target hydrocarbons in artificially spiked soils in the absence and presence of 5% (wet weight) of rhizosphere soil or root tissues are presented using graphing software package – SigmaPlot®, Version 12.5 (Systat Software Inc., USA).

3. Results

3.1. Mineralisation of ^{14}C–Hydrocarbons in Soil

The physicochemical and microbiological properties of the pasture soil are presented in Table 1. The ability of the indigenous soil microbial communities to mineralise ^{14}C–naphthalene, ^{14}C–phenanthrene, ^{14}C–hexadecane or ^{14}C–octacosane was measured in fresh artificially spiked soils (Figure 1 – Figure 4 [A & B] and Table 2) and 28 d pre–incubated artificially spiked soils (Figure 1 – Figure 4 [C & D] and Table 3) in the absence and presence of 5% (wet weight) of rhizosphere soil and root tissues of reed canary grass (*Phalaris arundinacea*), channel grass (*Vallisneria spiralis*), blackberry (*Rubus fructicosus*) and goat willow (*Salix caprea*).

The lag phases (the period of time before ^{14}C–hydrocarbons mineralisation exceeded 5%) in fresh (0 d) and 28 d pre–incubated artificially spiked soils varied between different treatment conditions (Table 2 & Table 3). In systems containing naphthalene, the lag phases varied from 3.27 ± 0.18 h (soil amended with reed canary grass root tissue) to 72.20 ± 0.04 h (unamanded soil) in fresh artificially spiked soils (0 d) and from 3.42 ± 0.10 h (soil amended with reed canary grass root tissue) to 4.15 ± 0.06 h (soil amended with channel grass root tissue) in 28 d pre–incubated artificially spiked soils. The lag phases in fresh artificially spiked soils amended with root tissues were significantly ($P < 0.001$) shorter compared to artificially spiked soils amended with rhizosphere soil and unamended soil. In systems containing phenanthrene, the lag phases varied from 30.20 ± 0.20 h (soil amended with channel grass root tissue) to 147.06 ± 2.17 h (unamanded soil) in fresh artificially spiked soils (0 d) and from 4.38 ± 0.14 h (soil amended with blackberry root tissue) to 6.00 ± 0.27 h (soil amended with reed canary grass root tissue) in 28 d pre–incubated artificially spiked soils. The lag phases in fresh artificially spiked soils amended with root tissues and rhizosphere soil were significantly ($P < 0.001$) shorter compared to unamended soil. Overall, the lag phases were significantly ($P < 0.001$) shorter in 28 d pre–incubated artificially spiked soils compared to fresh artificially spiked soils.

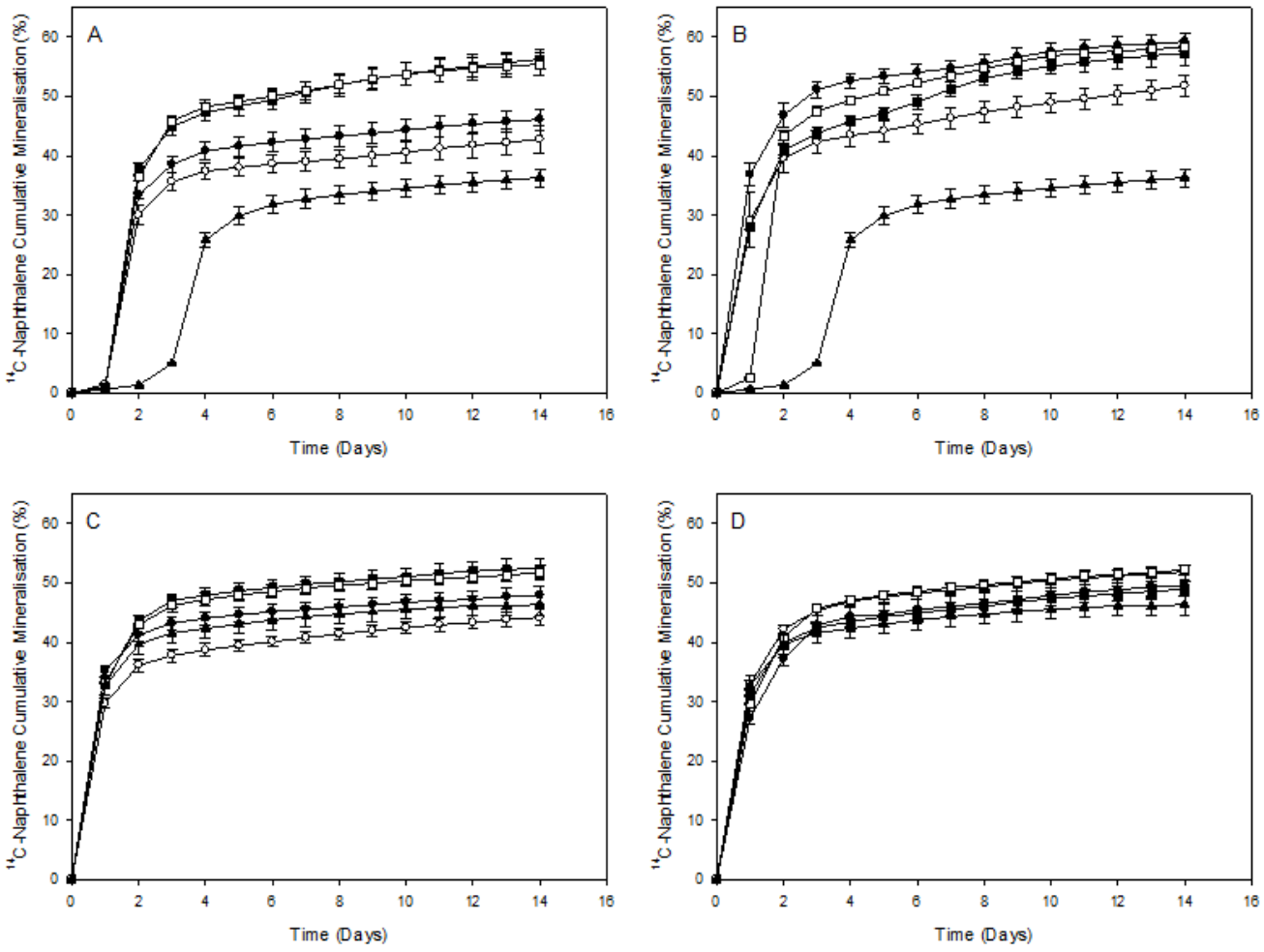

Figure 1. Microbial Mineralisation of ^{14}C–naphthalene in artificially spiked soil in the absence and presence of 5% (wet weight) of (A) rhizosphere or (B) root tissue at 0 d; and (C) rhizosphere or (D) root tissue after 28 d soil–organic contaminant pre–exposure. Plant saples used: Reed Canary Grass (●); Channel Grass (○); Blackberry (■); Goat Willow (□) or unamended soil (control) (▲). Data are presented as means ($n = 3$) and the errror bars are the standard error of mean (SEM)

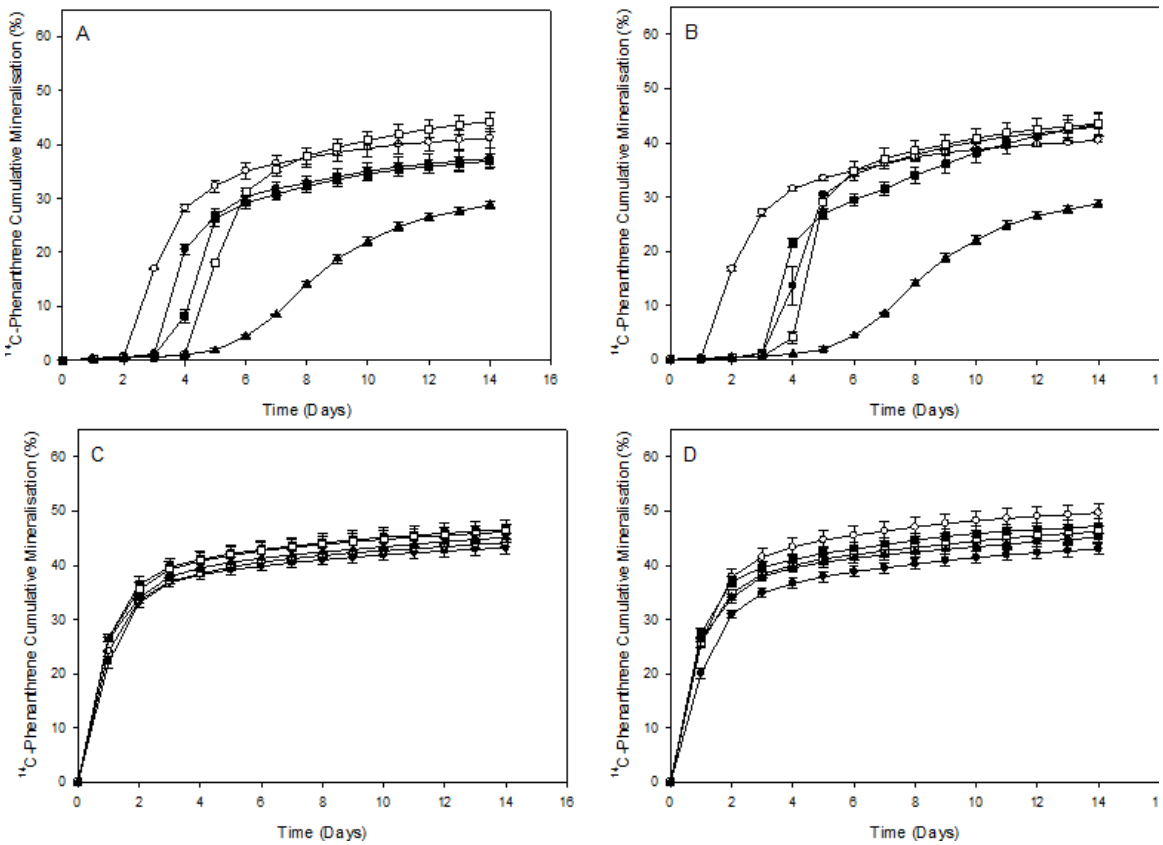

Figure 2. Microbial Mineralisation of ^{14}C–phenanthrene in artificially spiked soil in the absence and presence of 5% (wet weight) of (A) rhizosphere or (B) root tissue at 0 d; and (C) rhizosphere or (D) root tissue after 28 d soil–organic contaminant pre–exposure. Plant saples used: Reed Canary Grass (●); Channel Grass (○); Blackberry (■); Goat Willow (□) or unamended soil (control) (▲). Data are presented as means (n = 3) and the errror bars are the standard error of mean (SEM)

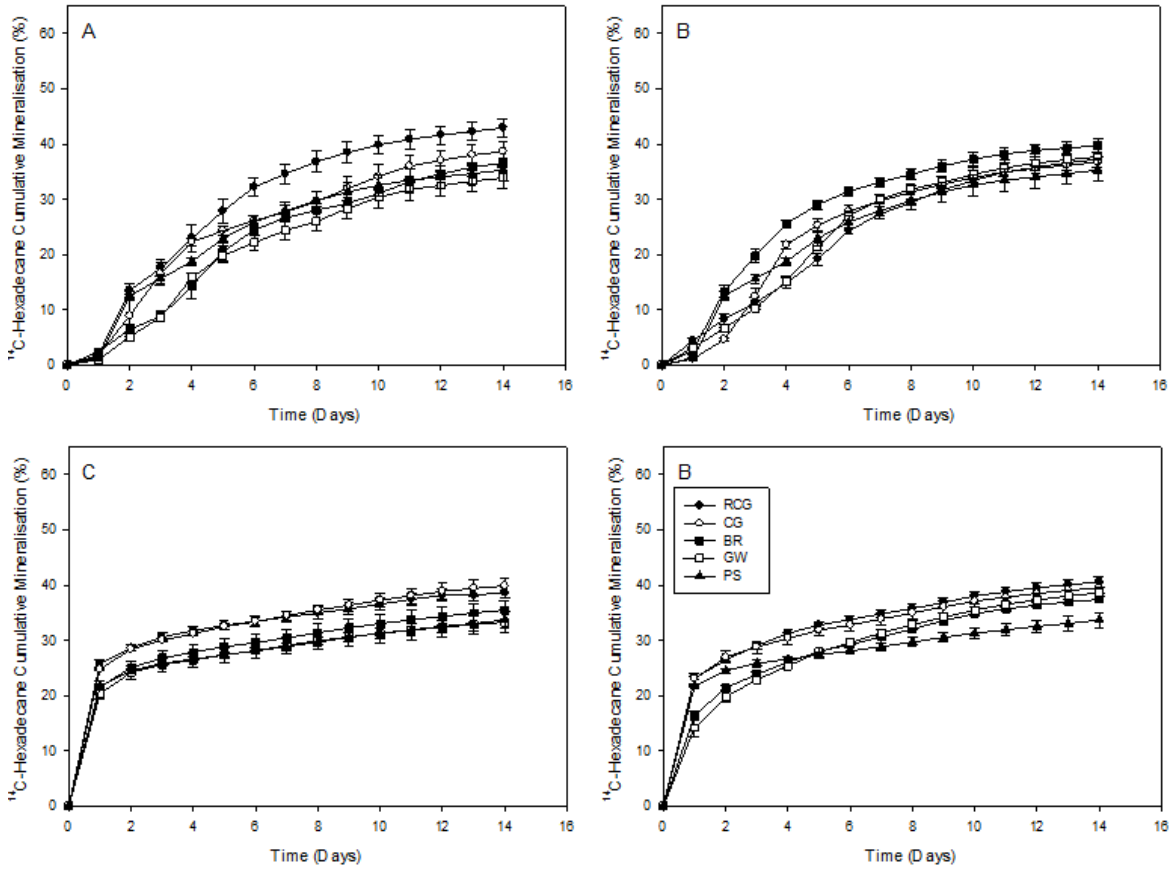

Figure 3. Microbial Mineralisation of ^{14}C–hexadecane in artificially spiked soil in the absence and presence of 5% (wet weight) of (A) rhizosphere or (B) root tissue at 0 d; and (C) rhizosphere or (D) root tissue after 28 d soil–organic contaminant pre–exposure. Plant saples used: Reed Canary Grass (●); Channel Grass (○); Blackberry (■); Goat Willow (□) or unamended soil (control) (▲). Data are presented as means (n = 3) and the errror bars are the standard error of mean (SEM)

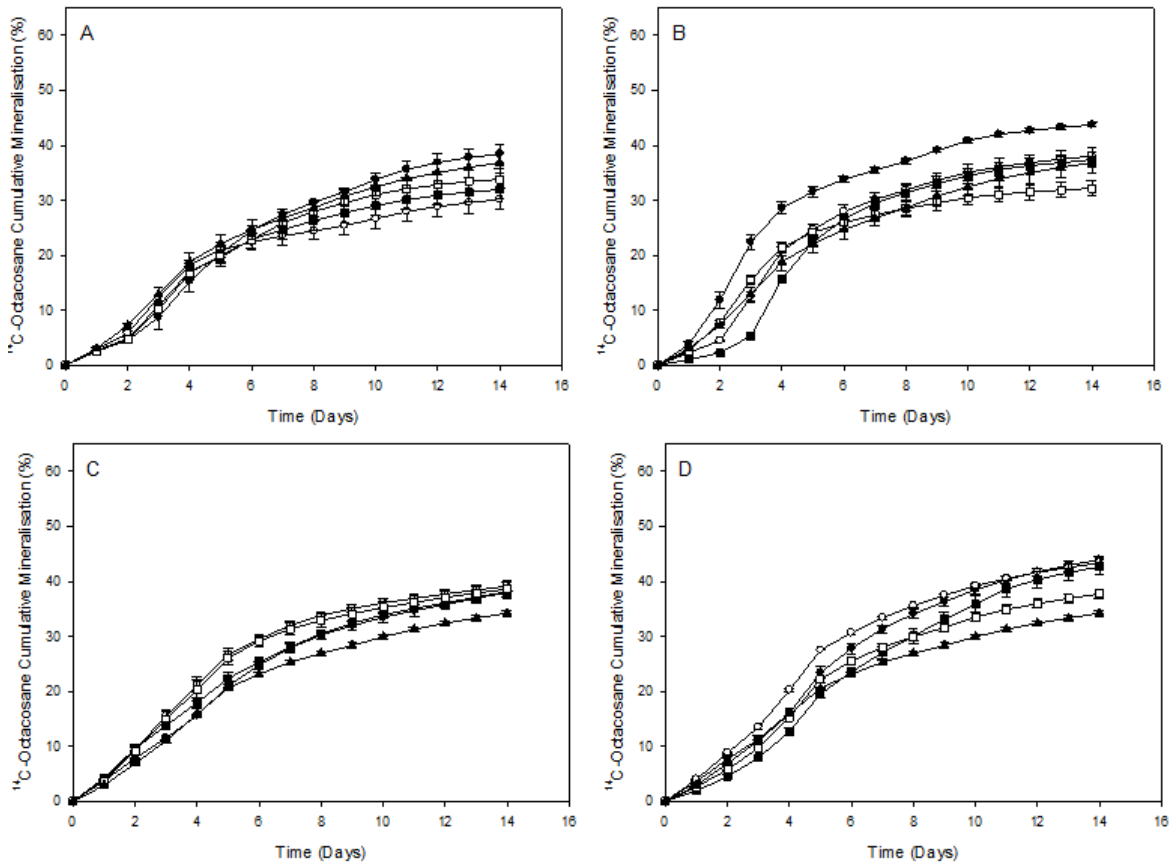

Figure 4. Microbial Mineralisation of ^{14}C–octacosane in artificially spiked soil in the absence and presence of 5% (wet weight) of (A) rhizosphere or (B) root tissue at 0 d; and (C) rhizosphere or (D) root tissue after 28 d soil–organic contaminant pre–exposure. Plant saples used: Reed Canary Grass (●); Channel Grass (○); Blackberry (■); Goat Willow (□) or unamended soil (control) (▲). Data are presented as means (n = 3) and the errror bars are the standard error of mean (SEM)

Table 1. Physicochemical and microbial characteristics of Myescough soil. Values are the mean (n = 3) ± standard errors of the mean (SEM).

Parameter		Value
pH in (dH_2O)		6.50 ± 0.08
Moisture content (%)		34.87 ± 0.89
Maximum water holding capacity (%)		38.03 ± 0.02
Elemental analysis		
	Total extractable organic carbon (%)	1.65 ± 0.01
	Total extractable carbon (%)	1.70 ± 0.09
	Total extractable nitrogren (%)	0.14 ± 0.01
	Soil organic matter (%)	2.71 ± 0.04
	Phosphorus (µg g^{-1})	997.00 ± 0.01
	C:N ratios	11.8:1
Particle analysis		
	Clay (%)	19.5 ± 0.70
	Silt (%)	20.0 ± 0.90
	Sand - Total (%)	60.4 ± 1.40
	Coarse sand	0.12 ± 0.01
	Medium sand	6.90 ± 0.10
	Fine sand	53.30 ± 0.60
Microbial analysis		
	Heterotrophs (CFU g^{-1})	$5.28 \times 10^4 \pm 0.00 \times 10^0$
	Hexadecane degraders (CFU g^{-1})	$6.24 \times 10^4 \pm 3.33 \times 10^4$
	Octocosane degraders (CFU g^{-1})	$3.05 \times 10^4 \pm 0.00 \times 10^0$
	Phenanthrene degraders (CFU g^{-1})	$4.04 \times 10^4 \pm 3.33 \times 10^4$
	Naphthalene degraders (CFU g^{-1})	$5.14 \times 10^4 \pm 3.33 \times 10^4$

Table 2. Microbial Mineralisation of ^{14}C–hydrocarbons (^{14}C–naphthalene, ^{14}C–phenanthrene, ^{14}C–hexadecane and ^{14}C–octacosane) in fresh artificially spiked soils (0 d) in the absence and presence of 5% (wet weight) of rhizosphere or root tissues. Values are the mean (*n*=3) ± standard error of the mean (SEM)

	Treatment conditions	Lag phase (h)			Maximum rate (% h^{-1})		
		Unamended Soil (Control)	Rhizosphere treatment	Root tissue treatment	Unamended Soil (Control)	Rhizosphere treatment	Root tissue treatment
Naphthalene	Bulk soil	72.20 ± 0.04			0.87 ± 0.05		
	Reed canary grass		26.93 ± 0.04	3.27 ± 0.18		1.35 ± 0.03	1.54 ± 0.08
	Channel grass		26.81 ± 0.83	4.79 ± 1.02		1.19 ± 0.07	1.21 ± 0.19
	Blackberry		26.69 ± 0.08	4.29 ± 0.08		1.54 ± 0.05	1.17 ± 0.02
	Goat willow		26.92 ± 0.03	25.54 ± 0.12		1.48 ± 0.02	1.70 ± 0.03
Phenanthrene	Bulk soil	147.06 ± 2.17			0.24 ± 0.02		
	Reed canary grass		76.74 ± 0.29	80.83 ± 2.52		0.81 ± 0.03	0.70 ± 0.15
	Channel grass		54.27 ± 0.12	30.72 ± 0.20		0.68 ± 0.01	0.68 ± 0.02
	Blackberry		86.08 ± 2.39	76.42 ± 0.32		0.75 ± 0.03	0.85 ± 0.06
	Goat willow		101.58 ± 0.04	94.13 ± 2.35		0.71 ± 0.01	1.04 ± 0.09
Hexadecane	Bulk soil	31.69 ± 0.69			0.47 ± 0.04		
	Reed canary grass		30.79 ± 0.69	28.59 ± 3.44		0.49 ± 0.05	0.22 ± 0.02
	Channel grass		47.19 ± 0.24	49.54 ± 1.16		0.32 ± 0.07	0.39 ± 0.07
	Blackberry		40.22 ± 3.69	29.53 ± 1.70		0.26 ± 0.04	0.45 ± 0.02
	Goat willow		50.42 ± 4.86	37.94 ± 1.65		0.30 ± 0.01	0.26 ± 0.03
Octacosane	Bulk soil	34.55 ± 2.51			0.25 ± 0.02		
	Reed canary grass		52.21 ± 5.62	28.02 ± 2.07		0.27 ± 0.01	0.44 ± 0.01
	Channel grass		41.81 ± 3.82	49.86 ± 1.15		0.27 ± 0.02	0.35 ± 0.01
	Blackberry		47.63 ± 2.68	68.99 ± 2.32		0.26 ± 0.01	0.43 ± 0.01
	Goat willow		47.69 ± 2.52	36.14 ± 0.69		0.28 ± 0.01	0.32 ± 0.03

	Treatment conditions	Cumulative extents (%)		
		Unamended Soil (Control)	Rhizosphere treatment	Root tissue treatment
Naphthalene	Bulk soil	36.31 ± 1.51		
	Reed canary grass		46.12 ± 1.76	59.24 ± 1.55
	Channel grass		42.79 ± 2.32	51.82 ± 1.72
	Blackberry		56.28 ± 1.75	57.32 ± 2.06
	Goat willow		55.46 ± 1.89	58.31 ± 0.16
Phenanthrene	Bulk soil	28.78 ± 0.63		
	Reed canary grass		37.43 ± 1.75	42.98 ± 0.45
	Channel grass		41.23 ± 1.75	40.47 ± 0.21
	Blackberry		36.95 ± 1.20	43.47 ± 2.08
	Goat willow		44.28 ± 1.80	43.54 ± 1.91
Hexadecane	Bulk soil	35.27 ± 2.07		
	Reed canary grass		42.93 ± 1.64	37.27 ± 2.07
	Channel grass		38.61 ± 1.92	36.73 ± 1.75
	Blackberry		36.57 ± 0.48	39.70 ± 1.15
	Goat willow		33.87 ± 1.88	37.69 ± 1.89
Octacosane	Bulk soil	36.88 ± 0.42		
	Reed canary grass		38.42 ± 2.02	43.75 ± 0.40
	Channel grass		30.20 ± 1.93	38.05 ± 1.47
	Blackberry		31.98 ± 1.71	37.40 ± 1.29
	Goat willow		33.84 ± 1.95	32.20 ± 1.43

Table 3. Microbial Mineralisation of ^{14}C–hydrocarbons (^{14}C–naphthalene, ^{14}C–phenanthrene, ^{14}C–hexadecane and ^{14}C–octacosane) in 28 d pre–incubated artificially spiked soils in the absence and presence of 5% (wet weight) of rhizosphere or root tissues. Values are the mean (*n=3*) ± standard error of the mean (SEM)

	Treatment conditions	Lag phase (h)			Maximum rate (% h^{-1})		
		Unamended Soil (Control)	Rhizosphere treatment	Root tissue treatment	Unamended Soil (Control)	Rhizosphere treatment	Root tissue treatment
Naphthalene	Bulk soil	3.86 ± 0.00			1.36 ± 0.07		
	Reed canary grass		3.42 ± 0.10	4.42 ± 0.18		1.47 ± 0.04	1.13 ± 0.05
	Channel grass		4.15 ± 0.06	3.58 ± 0.04		1.24 ± 0.04	1.37 ± 0.03
	Blackberry		3.66 ± 0.05	3.83 ± 0.07		1.37 ± 0.02	1.31 ± 0.02
	Goat willow		3.61 ± 0.01	4.05 ± 0.02		1.38 ± 0.00	1.23 ± 0.00
Phenanthrene	Bulk soil	4.52 ± 0.10			1.10 ± 0.02		
	Reed canary grass		5.45 ± 0.28	6.00 ± 0.27		0.92 ± 0.05	0.84 ± 0.04
	Channel grass		4.98 ± 0.02	4.72 ± 0.25		1.01 ± 0.00	1.08 ± 0.04
	Blackberry		4.50 ± 0.11	4.38 ± 0.14		1.11 ± 0.03	1.14 ± 0.04
	Goat willow		4.48 ± 0.03	4.82 ± 0.04		1.11 ± 0.01	1.07 ± 0.03
Hexadecane	Bulk soil	5.55 ± 0.07			0.90 ± 0.01		
	Reed canary grass		4.66 ± 0.12	5.20 ± 0.07		1.07 ± 0.03	0.96 ± 0.01
	Channel grass		4.83 ± 0.03	5.08 ± 0.21		1.03 ± 0.01	0.96 ± 0.01
	Blackberry		5.61 ± 0.31	7.35 ± 0.21		0.90 ± 0.05	0.68 ± 0.03
	Goat willow		5.63 ± 0.14	9.21 ± 1.34		0.85 ± 0.04	0.59 ± 0.06
Octacosane	Bulk soil	36.36 ± 0.14			0.20 ± 0.01		
	Reed canary grass		31.54 ± 0.55	32.85 ± 1.99		0.23 ± 0.02	0.30 ± 0.02
	Channel grass		30.14 ± 0.48	28.43 ± 0.62		0.24 ± 0.02	0.30 ± 0.01
	Blackberry		27.63 ± 1.22	51.77 ± 3.07		0.23 ± 0.01	0.29 ± 0.05
	Goat willow		28.83 ± 1.20	44.24 ± 1.19		0.24 ± 0.04	0.25 ± 0.03

	Treatment conditions	Cumulative extents (%)		
		Unamended Soil (Control)	Rhizosphere treatment	Root tissue treatment
Naphthalene	Bulk soil	46.39 ± 1.77		
	Reed canary grass		47.96 ± 1.49	49.39 ± 1.77
	Channel grass		44.20 ± 1.38	51.86 ± 1.19
	Blackberry		52.47 ± 1.72	48.96 ± 1.18
	Goat willow		51.78 ± 1.12	52.31 ± 0.84
Phenanthrene	Bulk soil	45.15 ± 0.87		
	Reed canary grass		43.23 ± 1.16	42.98 ± 1.01
	Channel grass		44.16 ± 0.74	49.68 ± 1.81
	Blackberry		46.58 ± 1.83	47.24 ± 1.30
	Goat willow		46.19 ± 1.49	46.21 ± 0.95
Hexadecane	Bulk soil	33.65 ± 1.37		
	Reed canary grass		38.68 ± 1.10	40.54 ± 1.01
	Channel grass		39.88± 1.43	39.47 ± 1.05
	Blackberry		35.49 ± 1.68	37.41 ± 0.43
	Goat willow		33.32 ± 1.80	38.62 ± 1.11
Octacosane	Bulk soil	34.21 ± 0.50		
	Reed canary grass		38.01 ± 1.06	43.92 ± 0.65
	Channel grass		39.12 ± 0.99	43.37 ± 0.67
	Blackberry		38.17 ± 1.01	42.76 ± 2.38
	Goat willow		38.62 ± 1.29	36.28 ± 0.96

In systems containing hexadecane, the lag phases varied from 28.59 ± 3.44 h (soil amended with reed canary grass root tissue) to 50.42 ± 4.86 h (soil amended with goat willow rhizosphere soil) in fresh artificially spiked soils and from 5.08 ± 0.21 h (soil amended with channel grass root tissue) to 9.21 ± 1.34 h (soil amended with goat willow root tissue) in 28 d pre–incubated artificially spiked soils. With exception of blackberry root tissue, reed canary grass rhizosphere soil and root tissue amendments, the lag phases in soils amended with rhizosphere soil and root tissues were significantly ($P < 0.05$) shorter compared to unamended soil. Overall, the lag phases were significantly ($P < 0.001$) shorter in 28 d pre–incubated artificially spiked soils compared to fresh artificially spiked soils. In systems containing octacosane, the lag phases varied from 28.02 ± 2.07 h (reed canary grass root tissue) to 68.99 ± 2.32 h (soil amended with blackberry root tissue) in fresh artificially spiked soils and from 27.63 ± 1.22 h (blackberry rhizosphere soil) to 51.77 ± 3.07 h (soil amended with blackberry root tissue) in 28 d pre–incubated artificially spiked soils. With the exception of reed canary grass root tissue amendment, the lag phases in fresh artificially spiked soils amended with rhizosphere soil or root tissues were not significantly different ($P > 0.05$) as compared to unamended soil. Notably, longer lag phases were measured in fresh artificially spiked soils amended with rhizosphere soils ($P < 0.001$) and in 28 d pre–incubated artificially spiked soils amended with root tissues (blackberry and goat willow) compared to unamended soils. Apart from systems containing octacosane, lag phases were significantly ($P < 0.001$) shorter in 28 d pre–incubated artificially spiked soils (Table 3) compared to those measured in fresh artificially spiked soils (Table 2).

The rates of ^{14}C–hydrocarbons (naphthalene, phenanthrene, hexadecane and octacosane) mineralisation in fresh artificially spiked soils and 28 d pre–incubated artificially spiked soils in the absence and presence of 5% (wet weight) of rhizosphere soil and root tissues are presented in Table 2 and Table 3. The rates of ^{14}C–naphthalene mineralisation were significantly ($P < 0.05$) faster in fresh artificially spiked soils amended with rhizosphere soil and root tissues compared to the unamended soil (Table 2). After 28 d pre–incubation, there were no significant ($P > 0.05$) differences in the maximum rates of ^{14}C–naphthalene mineralisation in artificially spiked soils in the absence and presence of rhizosphere soil and root tissues (Table 3). In systems containing ^{14}C–phenanthrene, the rates of mineralisation were significantly ($P < 0.001$) faster in fresh artificially spiked soils amended with rhizosphere soil and root tissues compared to the unamended soil (Table 2). However, there were no significant ($P > 0.05$) differences in the maximum rates of ^{14}C–phenanthrene mineralisation in artificially spiked soils in the absence and presence of rhizosphere soil and root tissues following 28 d pre–incubation (Table 3).

With the exception of reed canary grass rhizosphere soil and blackberry root tissue, rhizosphere soil and root tissues amendments significantly ($P < 0.001$) inhibited the maximum rates of ^{14}C–hexadecane mineralisation in fresh artificially spiked soils compared to the unamended soil (Table 2). Furthermore, blackberry and goat willow root tissues amendments significantly ($P < 0.001$) inhibited the maximum rates of ^{14}C–hexadecane mineralisation in 28 d pre–incubated artificially spiked soils compared to other treatment conditions (Table 3). Although the maximum rates of ^{14}C–octacosane mineralisation were only enhanced ($P < 0.05$) in fresh artificially spiked soils amended with root tissues (Table 2), amendment with rhizosphere soil and root tissues significantly ($P < 0.05$) enhanced the maximum rate of ^{14}C–octacosane mineralisation following 28 d pre–incubation compared to the unamended soil (Table 3). Depending on the physicochemical properties of the soil and contaminant concentration and/or bioavailability, the rates of indigenous microbial activities often increased following soil–organic contaminants pre–exposure.

The extents of ^{14}C–naphthalene mineralisation in fresh artificially spiked soils ranged from 42.79 ± 2.32 to 56.28 ± 1.75 % in systems amended with rhizosphere soil; 51.82 ± 1.72 to 59.24 ± 1.55 % in systems amended with root tissues, and 36.31± 1.51 % in unamended soil (Figure 1; Table 2). Enhanced extents of ^{14}C–naphthalene mineralisation were measured in soils amended with rhizosphere soil or root tissues ($P < 0.001$) compared to the unamended soil. The highest extents of ^{14}C–naphthalene mineralisation were measured in soils amended with root tissues of reed canary grass (59.24 ± 1.55 %) and goat willow (58.69 ± 0.16 %), while the lowest mineralisation extent was measured in soil amended with channel grass rhizosphere soil (42.79 ± 2.32 %). With the exception of soil amended with channel grass, there were no significant ($P > 0.05$) differences in the extents of ^{14}C–naphthalene mineralisation between rhizosphere soil and root tissues amended systems. After 28 d soil–contaminant pre–exposure, mineralisation extents ranged from 47.96 ± 1.49 to 52.47 ± 1.72 % in systems amended with rhizosphere soils; 48.96 ± 1.18 to 52.31 ± 0.84 % in systems amended with root tissues; and 46.39 ± 1.77 in unamended soil (Figure 1; Table 3). There were no significant ($P > 0.05$) differences in the extents of ^{14}C–naphthalene mineralisation between various amended soil treatments and unamended soil after 28 d soil–organic contaminant pre–exposure. However, there were subtle reductions in the extents of microbial mineralisation of ^{14}C–naphthalene in the 28 d pre–incubated spiked soil (Table 3) compared to fresh artificially spiked soil (Table 2).

The extents of ^{14}C–phenanthrene mineralisation in fresh artificially spiked soils ranged from 36.95 ± 1.20 to 44.28 ± 1.80 % in systems amended with rhizosphere soil; 40.47 ± 0.21 to 43.54 ± 1.91 % in systems amended with root tissues, and 28.78 ± 0.63 % in unamended soil (Figure 2; Table 2). Enhanced extents of ^{14}C–phenanthrene mineralisation were measured in soils amended with rhizosphere soil or root tissues ($P < 0.001$) compared to the unamended soil. In a direct comparison, there were no significant ($P > 0.05$) differences in the extents of ^{14}C–phenanthrene mineralisation in fresh artificially spiked soils amended with rhizosphere soil and root tissues. After 28 d soil–contaminant pre–exposure, extents of mineralisation ranged from 43.23 ± 1.16 to 46.19 ± 1.49 % in systems amended with rhizosphere soil; 42.98 ± 1.01 to 47.24 ± 1.30 % in systems amended with root tissues; and 45.15 ± 0.87 % in unamended soil (Figure 2; Table 3). There were no significant ($P > 0.05$) differences in extents of ^{14}C–phenanthrene mineralisation in amended artificially spiked soils and unamended soil at various time intervals (0 d and after 28 d pre–incubation).

The extents of ^{14}C–hexadecane mineralisation in fresh artificially spiked soils ranged from 33.87 ± 1.88 to 42.93 ± 1.64 % in systems amended with rhizosphere soil; 36.73 ± 1.75 to 39.70 ± 1.15 % in systems amended with root tissues, and 35.27 ± 2.07 % in unamended soil (Figure 3; Table 2). The extents of ^{14}C–hexadecane mineralisation in fresh artificially spiked soils amended with rhizosphere soil and root tissues were not significantly (P > 0.05) different compared to the unamended soil. After 28 d soil–contaminant pre–exposure, microbial mineralisation ranged from 33.32 ± 1.80 to 39.88 ± 1.43 % in systems amended with rhizosphere soil; 37.41 ± 0.43 to 40.54 ± 1.01 % in systems amended with root tissues, and 33.39 ± 1.37 % in unamended soil (Figure 3; Table 3). There were no significant (P > 0.05) differences in the extents of ^{14}C–hexadecane mineralisation between 28 d pre–incubated artificially spiked soils amended conditions and unamended soil. Overall, these results showed that contaminated soil amended with rhizosphere soil or root tissues did not enhance microbial mineralisation of ^{14}C–hexadecane by indigenous soil microbial communities.

The extents of ^{14}C–octacosane mineralisation in fresh artificially spiked soils ranged from 31.98 ± 1.71 to 38.42 ± 2.02 % in systems amended with rhizosphere soil; 32.20 ± 1.43 to 43.75 ± 0.40 % in systems amended with root tissues, and 36.88 ± 0.42 % in unamended soil (Figure 4; Table 2). The extents of ^{14}C–octacosane mineralisation in fresh artificially spiked soils amended with rhizosphere soil and root tissues were not significantly (P > 0.05) different compared to the unamended soil. However, the highest extent of mineralisation (43.75 ± 0.40 %) was measured in soil amended with reed canary grass root tissue, while the lowest extent of mineralisation (31.98 ± 1.71 %) was measured in fresh artificially spiked soil amended with channel grass rhizosphere soil. After 28 d soil–organic contaminant pre–exposure, microbial mineralisation of ^{14}C–octacosane ranged from 38.01 ± 1.06 to 39.12 ± 0.99 % in systems amended with rhizosphere soil; 36.28 ± 0.96 to 43.92 ± 0.65 % in systems amended with root tissues; and 34.21 ± 0.50 % in unamended soil (Figure 4; Table 3). There were no significant (P > 0.05) differences in the extents of ^{14}C–octacosane mineralisation between amended soils and unamended soil after 28 d pre–incubation. Overall, soils amended with rhizosphere soil or root tissues consistently displayed similar mineralisation patterns for systems containing ^{14}C–octacosane and ^{14}C–hexadecane.

Table 4. Microbial cell numbers of total heterotrophic and hydrocarbon–degrading microbes during ^{14}C–hydrocarbons mineralisation in fresh artificially spiked soils in the absence and presence of 5% (wet weight) of rhizosphere or root tissues. Values are the mean (n=3) ± standard error of the mean (SEM)

	Treatment conditions	Microbial cell numbers (CFU g^{-1}) in fresh artificially spiked soils (0 d)				
		Hydrocarbon–degraders (Unamended soil)	Heterotrophs (Rhizosphere soil)	Hydrocarbon–degraders (Rhizosphere soil)	Heterotrophs (Root tissues)	Hydrocarbon–degraders (Root tissues)
Naphthalene	Bulk soil	$6.06 \times 10^6 \pm 0.00 \times 10^0$				
	Reed canary grass		$8.99 \times 10^6 \pm 3.33 \times 10^4$	$6.06 \times 10^6 \pm 0.00 \times 10^0$	$1.21 \times 10^8 \pm 0.00 \times 10^0$	$1.76 \times 10^9 \pm 1.53 \times 10^7$
	Channel grass		$8.99 \times 10^6 \pm 3.33 \times 10^4$	$8.99 \times 10^7 \pm 3.33 \times 10^5$	$3.00 \times 10^8 \pm 5.77 \times 10^5$	$9.09 \times 10^8 \pm 0.00 \times 10^0$
	Blackberry		$1.49 \times 10^7 \pm 3.33 \times 10^4$	$6.06 \times 10^7 \pm 0.00 \times 10^0$	$6.06 \times 10^7 \pm 0.00 \times 10^0$	$9.09 \times 10^8 \pm 0.00 \times 10^0$
	Goat willow		$6.06 \times 10^7 \pm 0.00 \times 10^0$	$3.03 \times 10^7 \pm 0.00 \times 10^0$	$8.99 \times 10^8 \pm 3.33 \times 10^5$	$3.05 \times 10^9 \pm 3.33 \times 10^6$
Phenanthrene	Bulk soil	$5.56 \times 10^6 \pm 1.67 \times 10^4$				
	Reed canary grass		$8.99 \times 10^6 \pm 3.33 \times 10^4$	$5.96 \times 10^6 \pm 3.33 \times 10^4$	$1.21 \times 10^8 \pm 0.00 \times 10^0$	$9.09 \times 10^8 \pm 0.00 \times 10^0$
	Channel grass		$8.99 \times 10^6 \pm 3.33 \times 10^4$	$8.99 \times 10^7 \pm 3.33 \times 10^5$	$3.00 \times 10^8 \pm 5.77 \times 10^5$	$3.66 \times 10^9 \pm 3.33 \times 10^6$
	Blackberry		$1.49 \times 10^7 \pm 3.33 \times 10^4$	$5.96 \times 10^7 \pm 3.33 \times 10^5$	$6.06 \times 10^7 \pm 0.00 \times 10^0$	$3.15 \times 10^9 \pm 3.00 \times 10^7$
	Goat willow		$6.06 \times 10^7 \pm 0.00 \times 10^0$	$9.09 \times 10^7 \pm 5.77 \times 10^7$	$8.99 \times 10^7 \pm 3.33 \times 10^5$	$7.07 \times 10^8 \pm 6.67 \times 10^6$
Hexadecane	Bulk soil	$3.33 \times 10^6 \pm 5.77 \times 10^4$				
	Reed canary grass		$8.99 \times 10^6 \pm 3.33 \times 10^4$	$6.06 \times 10^7 \pm 0.00 \times 10^0$	$1.21 \times 10^8 \pm 0.00 \times 10^0$	$1.52 \times 10^9 \pm 5.77 \times 10^6$
	Channel grass		$8.99 \times 10^6 \pm 3.33 \times 10^4$	$8.99 \times 10^7 \pm 3.33 \times 10^5$	$3.00 \times 10^8 \pm 5.77 \times 10^5$	$9.09 \times 10^8 \pm 0.00 \times 10^0$
	Blackberry		$1.49 \times 10^7 \pm 3.33 \times 10^4$	$9.09 \times 10^7 \pm 0.00 \times 10^0$	$6.06 \times 10^7 \pm 0.00 \times 10^0$	$9.09 \times 10^8 \pm 5.77 \times 10^6$
	Goat willow		$6.06 \times 10^7 \pm 0.00 \times 10^0$	$5.86 \times 10^6 \pm 8.82 \times 10^5$	$8.99 \times 10^7 \pm 3.33 \times 10^5$	$6.26 \times 10^8 \pm 0.00 \times 10^0$
Octacosane	Bulk soil	$6.89 \times 10^6 \pm 8.82 \times 10^4$				
	Reed canary grass		$8.99 \times 10^6 \pm 3.33 \times 10^4$	$6.06 \times 10^7 \pm 0.00 \times 10^0$	$1.21 \times 10^8 \pm 0.00 \times 10^0$	$3.04 \times 10^9 \pm 3.33 \times 10^6$
	Channel grass		$8.99 \times 10^6 \pm 3.33 \times 10^4$	$9.09 \times 10^9 \pm 0.00 \times 10^0$	$3.00 \times 10^8 \pm 5.77 \times 10^5$	$9.39 \times 10^8 \pm 5.77 \times 10^6$
	Blackberry		$1.49 \times 10^7 \pm 3.33 \times 10^4$	$1.05 \times 10^8 \pm 6.67 \times 10^5$	$6.06 \times 10^7 \pm 0.00 \times 10^0$	$9.09 \times 10^8 \pm 0.00 \times 10^0$
	Goat willow		$6.06 \times 10^7 \pm 0.00 \times 10^0$	$3.03 \times 10^7 \pm 0.00 \times 10^0$	$8.99 \times 10^7 \pm 3.33 \times 10^5$	$9.80 \times 10^8 \pm 8.82 \times 10^6$

Table 5. Microbial cell numbers of total heterotrophic and hydrocarbon degrading microbes during ^{14}C–hydrocarbons mineralisation in 28 d pre–incubated artificially spiked soils in the absence and presence of 5% (wet weight) of rhizosphere or root tissues. Values are the mean (*n*=3) ± standard error of the mean (SEM)

	Treatment conditions	Microbial cell numbers (CFU g^{-1}) in 28 d pre–incubated soils				
		Hydrocarbon–degraders (Unamended soil)	Heterotrophs (Rhizosphere soil)	Hydrocarbon–degraders (Rhizosphere soil)	Heterotrophs (Root tissues)	Hydrocarbon–degraders (Root tissues)
Naphthalene	Bulk soil	$7.58 \times 10^6 \pm 5.77 \times 10^4$				
	Reed canary grass		$1.04 \times 10^7 \pm 6.67 \times 10^4$	$1.65 \times 10^8 \pm 3.33$ E5	$1.28 \times 10^8 \pm 3.33 \times 10^5$	$1.89 \times 10^9 \pm 1.20 \times 10^7$
	Channel grass		$1.03 \times 10^7 \pm 5.77 \times 10^4$	$1.03 \times 10^8 \pm 5.77$ E5	$2.98 \times 10^8 \pm 1.67 \times 10^6$	$9.80 \times 10^8 \pm 8.82 \times 10^6$
	Blackberry		$1.64 \times 10^7 \pm 5.77 \times 10^4$	$6.77 \times 10^7 \pm 3.33$ E5	$6.57 \times 10^7 \pm 1.67 \times 10^6$	$1.04 \times 10^9 \pm 3.33 \times 10^6$
	Goat willow		$7.07 \times 10^6 \pm 1.20 \times 10^5$	$5.25 \times 10^7 \pm 6.67$ E5	$8.99 \times 10^7 \pm 3.33 \times 10^5$	$3.00 \times 10^9 \pm 1.00 \times 10^7$
Phenanthrene	Bulk soil	$7.37 \times 10^6 \pm 3.33 \times 10^4$				
	Reed canary grass		$1.04 \times 10^7 \pm 6.67 \times 10^4$	$3.84 \times 10^7 \pm 8.82$ E5	$1.28 \times 10^8 \pm 3.33 \times 10^5$	$9.98 \times 10^8 \pm 3.33 \times 10^6$
	Channel grass		$1.03 \times 10^7 \pm 5.77 \times 10^4$	$9.60 \times 10^7 \pm 8.82$ E5	$2.98 \times 10^8 \pm 1.67 \times 10^6$	$3.73 \times 10^9 \pm 1.53 \times 10^7$
	Blackberry		$1.64 \times 10^7 \pm 5.77 \times 10^4$	$6.46 \times 10^7 \pm 8.82$ E5	$6.57 \times 10^7 \pm 1.67 \times 10^6$	$3.27 \times 10^9 \pm 5.77 \times 10^6$
	Goat willow		$7.07 \times 10^6 \pm 1.20 \times 10^5$	$1.00 \times 10^8 \pm 5.77$ E5	$8.99 \times 10^7 \pm 3.33 \times 10^5$	$7.47 \times 10^8 \pm 3.33 \times 10^6$
Hexadecane	Bulk soil	$3.54 \times 10^6 \pm 3.33 \times 10^4$				
	Reed canary grass		$1.04 \times 10^7 \pm 6.67 \times 10^4$	$5.66 \times 10^7 \pm 3.33 \times 10^5$	$1.28 \times 10^8 \pm 3.33 \times 10^5$	$1.59 \times 10^9 \pm 8.82 \times 10^6$
	Channel grass		$1.03 \times 10^7 \pm 5.77 \times 10^4$	$8.99 \times 10^7 \pm 3.33 \times 10^5$	$2.98 \times 10^8 \pm 1.67 \times 10^6$	$1.00 \times 10^9 \pm 5.77 \times 10^6$
	Blackberry		$1.64 \times 10^7 \pm 5.77 \times 10^4$	$9.90 \times 10^7 \pm 1.45 \times 10^6$	$6.57 \times 10^7 \pm 1.67 \times 10^6$	$9.44 \times 10^8 \pm 8.82 \times 10^6$
	Goat willow		$7.07 \times 10^6 \pm 1.20 \times 10^5$	$5.86 \times 10^7 \pm 8.82 \times 10^5$	$8.99 \times 10^7 \pm 3.33 \times 10^5$	$6.97 \times 10^8 \pm 5.77 \times 10^6$
Octacosane	Bulk soil	$7.07 \times 10^6 \pm 6.67 \times 10^4$				
	Reed canary grass		$1.04 \times 10^7 \pm 6.67 \times 10^4$	$6.06 \times 10^7 \pm 0.00$ E0	$1.28 \times 10^8 \pm 3.33 \times 10^5$	$3.12 \times 10^9 \pm 1.15 \times 10^7$
	Channel grass		$1.03 \times 10^7 \pm 5.77 \times 10^4$	$9.80 \times 10^7 \pm 3.33$ E5	$2.98 \times 10^8 \pm 1.67 \times 10^6$	$9.80 \times 10^8 \pm 3.33 \times 10^6$
	Blackberry		$1.64 \times 10^7 \pm 5.77 \times 10^4$	$1.06 \times 10^8 \pm 5.77$ E5	$6.57 \times 10^7 \pm 1.6\ 7 \times 10^6$	$9.70 \times 10^8 \pm 1.67 \times 10^6$
	Goat willow		$7.07 \times 10^6 \pm 1.20 \times 10^5$	$4.24 \times 10^7 \pm 5.77$ E5	$8.99 \times 10^7 \pm 3.33 \times 10^5$	$1.02 \times 10^9 \pm 8.82 \times 10^6$

3.2. Numbers of total heterotrophic and PAH–degrading bacteria

The numbers of total heterotrophic bacteria (THB) and hydrocarbon–degrading microbes in the artificially spiked soils amended with rhizosphere soil or root tissues were enumerated by standard microbiological technique (Table 4 & Table 5). In fresh artificially spiked soils, the numbers of total indigenous microbial communities in the amended soils ranged from 10^6 – 10^8 colony forming units per gram dry soil (CFU g^{-1}) for THB and 10^6 – 10^9 CFU g^{-1} for hydrocarbon–degrading microbial communities (Table 4). Microbial cell numbers of THB and hydrocarbon–degrading microbial communities of > 10^6 CFU g^{-1} were measured in the unamended soil. The numbers of CFU g^{-1} of THB and hydrocarbon–degrading microbial communities in the amended artificially spiked soils were in the similar range after 28 d pre–incubation compared to fresh artificially spiked soils. Although the CFU g^{-1} of THB and hydrocarbon–degrading microbial communities in unamended soil remained similar, the CFUs of hydrocarbon–degrading communities in artificially spiked soils amended with rhizosphere soil or root tissues significantly ($P < 0.001$) increased following 28 d soil–organic contaminants pre–exposure (Table 5). The highest numbers of hydrocarbon–degrading microbes > 10^9 CFU g^{-1} were measured in root tissues amended artificially spiked soils after 28 d soil–organic contaminants pre–exposure and microbial cell numbers in the amended soil and/or unamended soil increased following soil–organic contaminants pre–exposure.

4. Discussion

Root exudates, including organic compounds which are analogues of PAHs, may serve as nutrient sources for microbial growth and can stimulate the indigenous microbial degradation of organic contaminants in soil [12,43,44]. In this study, the rates and extents of ^{14}C–hydrocarbons (naphthalene, phenanthrene, hexadecane or octacosane) mineralisation in artificially spiked soils were monitored in the absence and presence of 5% (wet weight) of rhizosphere soil or root tissues (of reed canary grass, channel grass, blackberry and goat willow). The addition of rhizosphere soil or root tissues significantly enhanced ^{14}C–PAHs (phenanthrene and naphthalene), but did not stimulate ^{14}C–aliphatic hydrocarbons (hexadecane or octacosane) mineralisation in fresh artificially spiked soils. This has been attributed to the modification of microbial community and promotion of indigenous microbial activity caused by organic compounds presence in the rhizosphere soil or root tissues amendments. The root exudates components might have caused microbial

changes and enhanced degradative capacity of an indigenous microbial communities via a number of mechanisms, including shifts in catabolic gene expression, general metabolic status, and catabolic gene transfer [45,46]. In addition, changes in the physiological and metabolic capabilities could be attributed to physiochemical and biological changes caused by plant–derived organic compounds in the rhizosphere soil or root tissues amendments. Although the precise mechanism for this enhanced biodegradation remains unclear, it is obvious that the microbial changes might be associated with the active and effective carbon source supplemented by root exudates [44].

In this study, artificially spiked soils amended with root tissues of reed canary grass, blackberry and goat willow exhibited the highest extent of microbial degradation of hydrocarbons. The addition of rhizosphere soil or root tissues might have provided nutrient substrates for microbial growth and stimulated the desired microbial catabolic capabilities in the fresh artificially spiked soil [47]. It is possible that this was complicated by substrate interactions such as simultaneous biomass growth on multiple substrates [48]. PAH–analogous in the root exudates might have stimulated appropriate enzymatic pathways for microbial mineralisation of the PAHs in soil and the results support the findings of Miya and Firestone [17]. Evidence from previous studies have shown that the presence of monoterpenes stimulated the biodegradation of 2,4-dichlorophenol by indigenous soil microorganisms [36,49]. Several studies have linked increased hydrocarbon degradation in soil to plant root exudates and increases in rhizosphere associated microbial communities [20,21,50,51]. It has been reported that 3-ring PAHs such as phenanthrene demonstrated greater bioavailability because they are less hydrophobic than the high molecular weight compounds, and could act as sole carbon/energy source for a range of soil microbes [52]. In this present study, the enhanced microbial mineralisation of PAHs in fresh artificially spiked soils amended with rhizosphere soil or root tissues may be attributed to a combination of mechanisms rather than one single mechanism.

Changes in microbial cell numbers corresponded with enhanced microbial activity and growth of indigenous hydrocarbon–degrading microbes. This is consistent with the higher numbers of the hydrocarbon degraders enumerated in the amended artificially spiked soils and can be attributed to the rhizospheric microbes introduced with the rhizosphere soil or root tissues. It has been observed that root exudates supply organic compounds that serve as co–metabolites in microbial organic contaminant degradation or cometabolic biotransformation [26,28]. The addition of ground hybrid poplar roots produced a 165% increase in atrazine mineralisation [53] and this was primarily attributed to dehalogenase enzymes in the root tissues that have the capability to degrade atrazine [54]. Enhanced degradation through cometabolism of the benzo[a]pyrene by the rhizobacterium *Sphingomonas yanoikuyae* JAR02 *in vitro* in the presence of root extracts obtained from plant species, including mulberry (*Morus alba*) and hybrid willow (*Salix alba x matsudana*) has been reported by Rentz et al., *[55]*. A further explanation could be attributed to the effect of some of the bioactive compounds such as alkaloids, flavonoids, tannins, saponins, phenols and/or a cocktail of several other phytochemicals exuded by plant roots. In addition to root exudates that support the growth and activities of rhizosphere associated microbes [13]. Plant exudates may contribute to the enhanced mineralisation of organic contaminants through an increase in microbial density (more than 1–3 orders of magnitude than in non-vegetated or bulk soil), diversity and/or metabolic activity [56].

Although the degree of enzyme release into soils and sediments remains poorly understood [54], the presence of phytochemical compounds in rhizosphere soil or root tissue in sufficient quantities might have primed specific biodegradation activities or promote selective degradation capacity of the indigenous soil microbes. However, it is widely accepted that the rates and extents of degradation for different aromatic hydrocarbons are known to differ as a result of physical-chemical properties such as molecular size, structure, hydrophobicity and solubility [57]. In this study, ^{14}C–aliphatic hydrocarbon mineralisation in soil amended with rhizosphere soil or root tissue may be due to the preferential or co–utilisation of the additional carbon supplied in the amendments over the target substrate. It has been reported that aliphatic and aromatic hydrocarbons such as flavonoids [58] and phenanthrene derivatives such as retene and nudol [59], occur naturally in plant materials. It is possible that plant organic compounds from amendment influence soil microbial activity by providing co–substrates for biomass growth [48]. According to Read et al. [60], plant roots release phospholipid surfactants that modify the physical and chemical properties of soil. In this study, associated organic compounds from rhizosphere soil or root tissues amendment might have affected the solubility and/or bioavailability of aliphatic hydrocarbons during the slurry biodegradation. However, alkanes such as *n*–hexadecane have a log K_{ow} of approximately 9.1, a reported solubility of up to 0.0263 mg l^{-1} [61], and as discrete compounds are unlikely to be effectively mineralised by the indigenous soil microbes. Pre–exposure of soil microorganisms to organic contaminant, but not amendment with rhizosphere soil or root tissues, appeared to be the main factor that affect ^{14}C–aliphatic hydrocarbon mineralisation.

5. Conclusion

This study demonstrated that the addition of rhizosphere soil or root tissues of reed canary grass (Phalaris arundinacea), channel grass (Vallisneria spiralis), blackberry (Rubus fructicosus) and goat willow (Salix caprea) significantly enhanced 14C–PAHs mineralisation, but did not stimulate microbial mineralisation of 14C–aliphatic hydrocarbons in fresh artificially spiked soils. The enhanced indigenous microbial degradation of PAHs in fresh artificially spiked soils amended with rhizosphere soil or root tissues may be attributed to a combination of mechanisms (involving contribution of root exudates) rather than one single mechanism. Findings from this study has provided further insights into the understanding of enhanced microbial mineralisation of PAHs in rhizosphere soil and potential effects associated with plant–derived organic compounds from rhizosphere soil or root tissues during phytoremediation of PAHs contaminated soil. This study further confirmed the finding of Stroud et al. [62], who reported that lower molecular weight (LMW)

PAHs are mineralised faster than those of a higher molecular weight (HMW) due to the fact that LMW PAHs are more suitable as sole carbon sources for microbial communities. Although pre–exposure of indigenous soil microorganisms to hydrocarbons decreased the lag phases and increased the initial rates of microbial mineralisation, addition of plant secreted chemicals may have practical application for remediation of petroleum–contaminated soils. The results are important for development of bioremediation strategies for PAHs contaminated soil [47] and further research is required to identify specific root exudate components which enhanced microbial degradation of organic contaminants in soil.

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