

# MULTISPECIES AND MONOCULTURE RHIZOREMEDIATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) FROM THE SOIL

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## I. INTRODUCTION

Phytoremediation, the use of plants and associated microorganisms to degrade or immobilize contaminants in soil and groundwater, is increasingly being considered for rehabilitating moderately contaminated soils. This technology represents a potential low-cost, effective, and low-maintenance alternative for waste management (Aprill and Sims, 1990). The mechanisms of phytoremediation for hazardous organic contamination in soil include, direct plant uptake, microbial degradation stimulated by plant roots, cometabolism of contaminants in the rhizosphere and adsorption to humic or organic matter (Chen *et al.*, 2003; Cunningham and Ow, 1996; Schnoor *et al.*, 1995).

Laboratory and greenhouse experiments on plant-mediated dissipation of polycyclic aromatic hydrocarbons (PAHs) have concentrated mainly on the use of monoculture rhizoremediation of PAHs from the soil (Aprill and Sims, 1990; Günther *et al.*, 1996; Lee and Banks, 1993; Reilly *et al.*, 1996; Walton *et al.*, 1994). In these studies, the extent of organic pollutant removal in planted soil has been significantly greater than in nonplanted soil. However, the information about the effectiveness of multispecies rhizoremediation of PAHs is lacking. The use of soil microcosms with mixed planted species has the potential to increase soil heterogeneity (Angers and Caron, 1998) and microbial diversity, which can improve the microbial competence of the soil bacteria for effective pollutant removal.

In this study, we evaluated the effectiveness of both monoculture rhizoremediation and multispecies rhizoremediation of PAH-contaminated soil. In addition, we evaluated the functional diversity of the PAH-contaminated and noncontaminated rhizosphere and nonrhizosphere soil using community-level physiological profiles.

## II. MATERIALS AND METHODS

### A. Chemicals

All solvents (dichloromethane 99% and trichloromethane 99%) and PAH (Naphthalene 98%, fluorene 98%, and pyrene 98%) were purchased from Sigma-Aldrich, South Africa.

### B. Soil

The predominantly sandy loam soil used in the experiments was taken from the Pretoria Campus of the Council of Scientific and Industrial Research (CSIR), South Africa. The artificial contamination of the soil with PAH was done as described by Leyval and Binet (1998). The PAHs

were first dissolved in trichloromethane before being mixed with 1% of the total soil to be polluted. Trichloromethane was allowed to volatilize under fumehood and the amended soil was mixed thoroughly with the remaining 99% of the soil. After mixing, three samples of the artificially contaminated soil were collected in sealable glass containers for PAHs analysis. The concentration of the individual PAHs in the soil averaged 300 mg/kg soil. Further soil sampling and storage of the rhizosphere and nonrhizosphere soil in the experiments were done as described by Wollum (1982). The rhizosphere soil (soil that adheres to the roots) was collected in sterile bags by gently shaking loose soil from the intermingled roots of the planted treatments. This rhizosphere soil was used for determining the community-level physiological profiles and for PAH analysis. The nonrhizosphere soil was collected from the pots and mixed thoroughly before further analysis. Analyses were made within 24 h of sampling. However, in cases where this was not feasible, the soil samples were stored overnight at 4°C.

### **C. Plants**

The seeds of *Brachiaria serrata* (Velvet signal grass) were bought from Agricol Pty Ltd, South Africa, and those of *Eleusine coracana* (African millet) were obtained from Plant Genetic Resources Directorate (Agriculture Department, South Africa). *B. serrata* is a perennial tufted grass with shrub-like growth form. *E. coracana* is a robust annual tufted grass with slated culms and has an exceptionally dense and strong root system. The grass seeds were sown in trays (38 cm x 38 cm) containing the soil. The trays were incubated at room temperature in the greenhouse with natural day-night cycles until germination of both plant seeds occurred. No supplemental lighting was supplied. However, the average natural cycle was 9 h of daylight. The room temperature in the greenhouse averaged 28°C during the period of incubation. The grass seedlings were used in the phytoremediation experiment (vegetated microcosms).

### **D. Vegetated and Nonvegetated Microcosms**

Plants mediated removal of PAH was evaluated using monoplanted and multiplanted soil microcosms. The soil was artificially contaminated with PAHs as described above. The concentration of each PAH in the soil was 300 mg/kg soil. This “time zero” concentration is the average of the individual PAHs detected. The monoplanted and multiplanted treatments were prepared as shown in Table 1. Six hundred grams of each soil preparation (Table 1) were placed in 10-cm pots with saucers for leachate collection. The grass seedlings were planted in different pots as shown in Table 1. The density of the plants was one plant per pot for the monocultured treatments, while two plants (as shown in Table 1) were used for the multispecies treatment. The pots were placed in the greenhouse at room temperature and natural day-night cycles. No supplemental lighting was supplied. However, the average natural cycle was 9 h of daylight. The room temperature in the greenhouse averaged 28°C during the period of incubation. Two hundred millilitres of water was used to water the plants every 2 d. In instances where leachates were produced, the leachate was used to water the same pots. Each treatment had 11 pots and 2 pots for each treatment were sacrificed after 2, 6, and 10 wk of incubation to determine concentrations of PAHs in the soil. ANOVA (Analysis of Variance) was used to determine the difference between the treatments.

### **E. Chemical Analysis**

Residual PAH in treatments T0-T3 were quantified as described by Maila and Cloete (2002). PAHs were extracted from 25 g soil of each treatment using dichloromethane. PAHs were quantified after extraction with dichloromethane, using a Varian Saturn 2000 Ion Trap Gas

Chromatography/Mass Spectrometer equipped with a Chrompack CP-SIL 8CB-MS (5% phenyl) Fused Silica Capillary Column (30 m\* 0.25 mm\* 0.25 / $\mu$ m). The detector was tuned according to EPA 8270C using DFTPP. Injector temperature was 230°C, oven temperature program: 30°C (6 min), 10°C/min, 300°C (7 min). Analyses were done in triplicate.

**Table 1** Treatments used in the experiments

Treatments	Additions/Preparations
T0 (Control)	Soil + PAHs
T1 (CSEC)	Soil + PAHs + <i>E.corocana</i>
T2 (CSBS)	Soil + PAHs + <i>B.serrata</i>
T3 (CSBSEC)	Soil + PAHs + <i>E.corocana</i> + <i>B.serrata</i>
T4 (UCS)	Soil
T5 (UCSEC)	Soil + <i>E.corocana</i>
T6 (UCSBSEC)	Soil $\pm$ <i>B.serrata</i> $\pm$ <i>E.corocana</i>

## F. Community-Level Physiological Profiles (CLPPs)

The microbial community level physiological profiles of the different treatments shown in Table 1 was done using the soil samples sacrificed after 4 wk of incubation. This period was deemed to be sufficient to allow the acclimatisation of the plants in the contaminated and noncontaminated soils. In addition, the PAH dissipation analysis were also done (as described above) using the samples sacrificed after 4 wk of incubation (week 4 PAH dissipation results not included). For CLPP determination, three pots per treatment were used. The rhizosphere and nonrhizosphere soils were sampled as described above and microbial suspensions were prepared from the soil for Biolog<sup>TM</sup> inoculation as described by Wünsche et. al. (1995). Ten grams of soil and 100 ml of 0.2 % tetra-sodium pyrophosphate solution were shaken in an Erlenmeyer flask for 30 min. on a rotary shaker at 140 rpm. The mixture was allowed to settle for 5 min. and the supernatant used for serial dilutions in a physiological saline solution. After appropriate dilutions in sterile saline solution, the cell suspensions were used to determine the number of culturable heterotrophs and to inoculate BIOLOG<sup>TM</sup> GN micro plates. The number of culturable heterotrophs (results not included), expressed as CFU, was determined by spreading 0.1 ml cell suspension on to a nutrient agar (Biolab Diagnostics Pty Ltd., South Africa) medium, amended with cycloheximide (200  $\mu$ g/ml) to suppress fungal growth. Plate counting was done in triplicate and incubation was at 28°C for 24 h. Dilutions giving the same number of culturable heterotrophs were used for Biolog<sup>TM</sup> inoculation.

To obtain the metabolic fingerprints of the microbial communities in different treatments (Table 1), three replicate (three pots per treatment) of all the soil extracts were inoculated in BIOLOG<sup>TM</sup> GN microtiter plates (Biolog Inc., Hayward, CA) containing 95 different sole-carbon sources and a control without a carbon source. 100  $\mu$ L of each soil extract was added to each well. The BIOLOG<sup>TM</sup> GN plates were incubated at 28°C and readings done using a Bio-Tek Elx800 (Bio-Tek Instruments Inc., IL, USA) micro plate reader at 600 nm after 24,48, and 72 h. Statistical analyses were done using STATISTICA for Windows release 5.1.

## III. RESULTS

Plant-mediated PAH dissipation was evaluated using monoplanted and multiplanted soil microcosms. PAH dissipation was higher in the planted soils compared to the nonplanted soil (Tables 2–4). There was, on average, a 97% reduction of naphthalene in planted soils compared

to 63% in the nonplanted soil. The dissipation of naphthalene and fluorene was higher in the multiplanted soil compared to the mono- and nonplanted soil (Tables 2 and 3). The concentration of naphthalene was undetectable in the multiplanted treatment compared to 96% reduction in the mono- and nonplanted treatments and 63% reduction in the non-planted control after 10 wk of incubation. There was a 96% reduction in fluorene from the multiplanted treatment compared to 81% reduction in the mono- and nonplanted treatment with *E. corocana* and 47% of the control

**Table 2** Naphthalene concentrations (mg/kg soil) in monocultured and multiplanted soil treatments (average  $\pm$  std. dev)

Weeks of Incubation	Control (T0)	CSEC (T1)	CSBS (T2)	CSECBS (T3)
2	293 $\pm$ 0.632	224 $\pm$ 0.089	202 $\pm$ 1.032	198 $\pm$ 0.019
6	248 $\pm$ 0.059	110 $\pm$ 1.112	98 $\pm$ 0.221	66 $\pm$ 1.532
10	111 $\pm$ 0.632	12 $\pm$ 0.321	9 $\pm$ 1.209	0

**Table 3** Fluorene concentrations (mg/kg soil) in monocultured and multiplanted soil treatments (average  $\pm$  std. dev)

Weeks of Incubation	Control (T0)	CSEC (T1)	CSBS (T2)	CSECBS (T3)
2	296 $\pm$ 0.089	241 $\pm$ 0.087	246 $\pm$ 0.009	236 $\pm$ 0.127
6	235 $\pm$ 0.635	178 $\pm$ 1.612	164 $\pm$ 0.112	110 $\pm$ 0.255
10	160 $\pm$ 1.541	56 $\pm$ 0.065	38 $\pm$ 2.872	10 $\pm$ 1.432

treatment. However, there was no significant difference ( $P < 0.01$ ) in the dissipation of pyrene in both the mono- and multiplanted treatments (Table 4). There also was no significant difference between the dissipation of PAHs in the treatment with *Brachiaria serrata* and the treatment with *Eleusine corocana* (Tables 2–4).

As the development of microbial communities inhabiting the root zone is influenced by plant species, it was expected that the dissipation of PAHs in treatments vegetated by different plant species would be different. However, this was not observed, as there was no significant difference ( $P < 0.01$ ) in PAH dissipation in treatments with *B. serrata* (T2) and treatment with *E. corocana* (T1).

In addition, as plants have the potential to increase soil heterogeneity and soil microbial diversities, it was expected that the treatment with “multiplants” would have a higher PAH dissipation compared to mono- and nonplanted (T1 and T2) treatments. This was observed as both naphthalene and fluorene dissipation was significantly higher ( $p < 0.05$ ) in the multiplanted treatments compared to the mono- and nonplanted treatments. However, different results were obtained for pyrene degradation.

## CLPPs

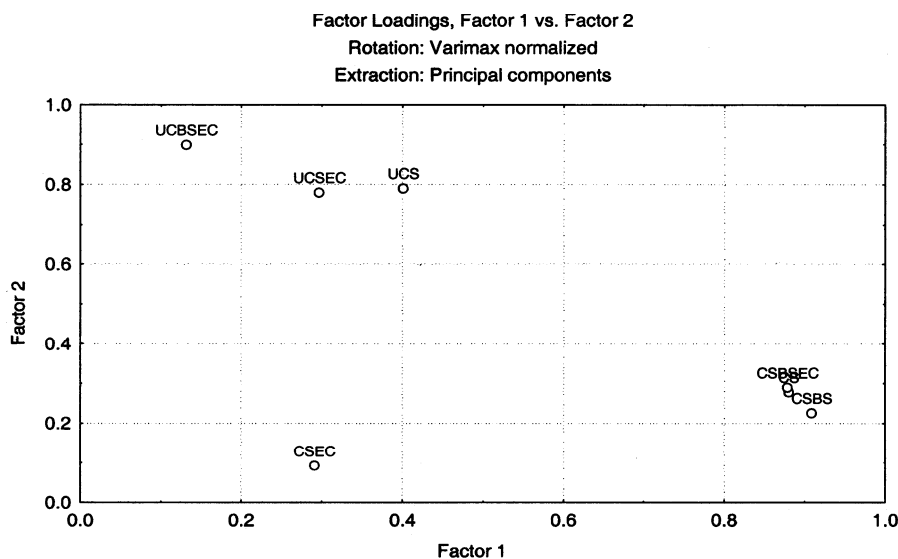
Heterotrophic microbial communities were evaluated during phytoremediation of the PAHs by determining the metabolic fingerprints of the different treatments. Biolog plates with 95 different substrates were used to determine the community level physiological profiles of the different treatments. Both principle component analysis PCA and cluster analysis were used to evaluate the difference in substrate utilization patterns of the different

treatments. PCA was performed to characterize the correlation between treatments, taking into account the absorbance values for all 96-response wells. Two principal factors were isolated from the individual patterns (Figure 1), which explained 63% of the variation. For the samples, factor one was related to the absorbance values for the wells, while factor 2 was related to the incubation time.

The metabolic fingerprints of the PAH-contaminated soils (CS, CSBS, CSBSEC, CSEC) were not closely related to the metabolic fingerprints of the noncontaminated soils, (UCS, UCSEC, UCBSEC; Figure 1). The metabolic fingerprint of the PAH-contaminated soil (CS) was more closely related to the contaminated monoplanted treatment with *Brachiaria serrata* (CSBS) and contaminated multiplanted soil with *Brachiaria serrata* and *Eleusine corocana* (CSBSEC) than to other treatments (Figure 1). The uncontaminated

**Table 4** Pyrene concentrations (mg/kg soil) in monocultured and multiplanted soil treatments (average  $\pm$  std. Dev)

Weeks of Incubation	Control (T0)	CSEC (T1)	CSBS (T2)	CSECBS (T3)
2	298 $\pm$ 0.283	262 $\pm$ 0.064	258 $\pm$ 0.832	261 $\pm$ 0.432
6	266 $\pm$ 0.642	203 $\pm$ 1.412	218 $\pm$ 1.414	198 $\pm$ 0.432
10	255 $\pm$ 0.565	166 $\pm$ 1.632	128 $\pm$ 0.876	117 $\pm$ 1.555



**Figure 1** Metabolic diversity of PAH-contaminated and noncontaminated rhizosphere and nonrhizosphere soils. UCS—uncontaminated soil, CS—contaminated soil, CSBS—contaminated soil with *Brachiaria serrata*, UCSEC—Uncontaminated soil with *Eleusine corocana*, UCBSEC—uncontaminated soil with *Brachiaria serrata* and *Eleusine corocana*, CSBSEC—contaminated soil with *Brachiaria serrata* and *Eleusine corocana*.

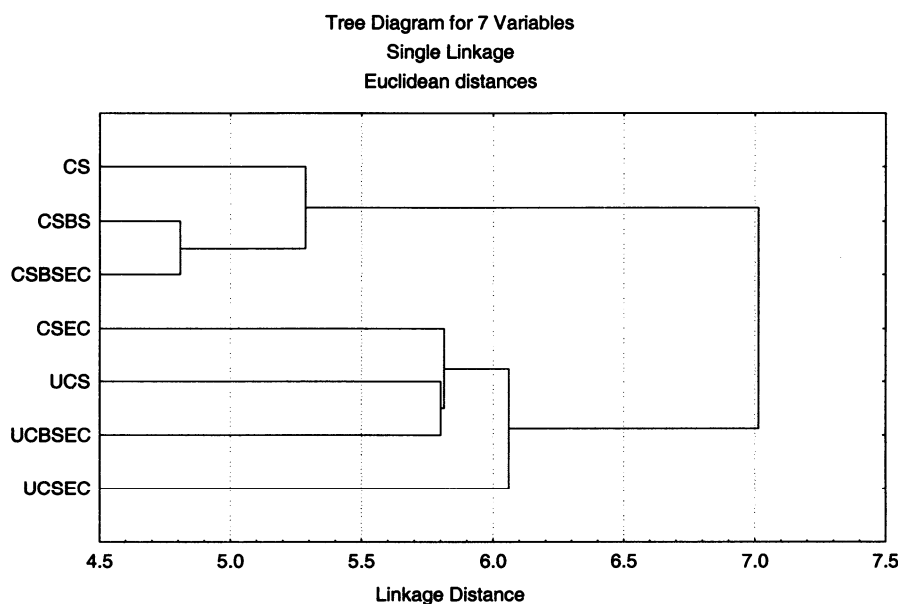
soils were more “closely” related to each other than to the PAH-contaminated soils.

The metabolic fingerprints of the different treatments were further evaluated using hierarchical clustering (Figure 2). In a dendrogram, the contaminated soils (CS, CSBS, and CSBEC) were clustered together while CSEC was clustered with the uncontaminated soils. Both PCA and cluster analysis indicated that the metabolic fingerprints of PAH contaminated soils (with the exception of CSEC) were closely related, as were the metabolic fingerprints of the uncontaminated soils.

As plants have the potential to increase soil heterogeneity and possibly soil diversity, it was expected that treatments with more plants will have high microbial competence, reflected by higher PAH dissipation and higher number of Biolog substrates (Tables 5 and 6) used by the multiplanted treatments compared to mono-planted treatments. Higher PAH dissipation was only evident with naphthalene and fluorene (Tables 2 and 3), while there was no significant difference in the amount of Biolog substrates (Table 6) used by the multiplanted and mono-planted contaminated treatments after 72 h of incubation. However, more Biolog substrates were used by microorganisms in the planted treatments compared to the nonplanted treatments (Table 6).

#### IV. DISCUSSIONS AND CONCLUSION

This study investigates plant-mediated PAH dissipation using mono-planted and multiplanted soil microcosms. PAH dissipation was higher in the planted soils compared to



**Figure 2** Cluster analysis of the PAH-contaminated and noncontaminated rhizosphere and nonrhizosphere soils.

the nonplanted control soil. This finding was in line with other findings, which indicated enhanced PAHs dissipation in vegetated soils compared to nonvegetated soil (Aprill and Sims, 1990; Reilly *et al.*, 1996). According to Muratova *et al.* (2003), significant PAH dissipation is attained in the vegetated soils as plants stimulate the rhizosphere micro flora, which degrade the pollutants.

The dissipation of naphthalene and fluorene was higher in the multiplanted soil compared to the monoplanted and nonplanted control soil. However, there was no significant difference in the dissipation of pyrene in the mono- and multiplanted treatment. This was not surprising, as the rate of PAH degradation is inversely proportional to the number of rings in the PAH molecule (Cerniglia, 1992; Cerniglia and Heitkamp, 1989). In addition, the duration of incubation was probably not sufficient for the complete dissipation of pyrene in both treatments or the lack of pyrene biodegraders was responsible for this difference.

There was also no significant difference between the dissipation of PAHs in the treatment with *Brachiaria serrata* and the treatment with *Eleusine corocana*. As plant species influence the development of microbial communities inhabiting the root zone (Rovira, 1956, 1959), it was expected that the dissipation of PAHs in treatments vegetated by different plant species will be different. This expectation emanated from the fact that the two grasses have slightly different root structure and also due to the probable difference in the root exudates, which may influence the “competency” of the rhizosphere microbial community inhabiting the plants. However, this was not realized, as there was no significant difference in PAH dissipation between treatments with *B. serrata* and treatment with *E. corocana*. This finding was in contrast to findings by Muratova *et al.* (2003), who reported differences in the degradation of PAH with different plant species. However, alfalfa and reed species were used in their study, while different grass species were used in this study.

The data on enhanced dissipation of both naphthalene and fluorene in multiplanted treatments compared to monoplanted treatments suggests that phytoremediation can be enhanced by “bioaugmenting” moderately contaminated soil with multiplants instead of monocultures.

**Table 5** Carbon sources in biolog GN microplates

<b>Biolog carbon sources</b>		
<b>Carboxylic acids</b>	<b>Amino acids</b>	<b>Carbohydrates</b>
Propionic acid	D-Alanine	D-Fructose
Quinic acid	L-Alanine	D-Fucose
D-Saccharic acid	L-Alanyl-Glycine	D-Galactose
Sebacic acid	D-Serine	Gentiobiose
Succinic acid	L-Serine2	$\alpha$ -D-Glucose
Acetic acid	L-Threonine	m-Inositol
Cis-Aconitic acid	D,L-Carnitine	$\alpha$ -Lactose
Citric acid	L-Asparagine	N-Acetyl-D-galactosamine
Formic acid	L-Aspartic acid	N-Acetyl-D-glucosamine
D-Galactonic acid lactone	L-Glutamic acid	Adonitol
D-Galacturonic acid	Glycyl-L-Aspartic acid	L-Arabinose
D-Gluconic acid	Glycyl-L-glutamic acid	D-Arabitol
D-Glucosaminic acid	L-Histidine	Cellobiose
D-Gluconic acid	Hydroxy-L-proline	<i>i</i> -Erythritol
$\alpha$ -Hydroxybutyric acid	L-Leucine	Lactulose
$\beta$ -Hydroxybutyric acid	L-Ornithine	Maltose
$\gamma$ -Hydroxybutyric acid	L-Phenylalanine	D-Mannitol
$\rho$ -Hydroxyphenylacetic acid	L-Proline	D-Mannose
Itaconic acid	L-Pyroglutamic acid	D-Melibiose
$\alpha$ -Ketobutyric acid	$\gamma$ -Aminobutyric acid	$\beta$ -Methylglucoside
$\alpha$ -Ketoglutaric acid	<b>Amines</b>	Psicose
$\alpha$ -Ketovaleric acid	Phenylethylalamine	D-Raffinose
D,L-Lactic acid	2-Aminoethanol	L-Rhamnose
Malonic acid	Putrescine	D-Sorbitol
<b>Phosphorylated chemicals</b>	<b>Aromatic chemicals</b>	Sucrose
Glucose-1-phosphate	Inosine	D-Trehalose
Glucose-6-phosphate	Urocanic acid	Turanose
D,L- $\alpha$ -Glycerol phosphate	Thymidine	Xylitol
<b>Alcohols</b>	Uridine	<b>Polymers</b>
2,3-Butanediol	<b>Brominated chemicals</b>	Dextrin
Glycerol	Bromosuccinic acid	Glycogen
<b>Amides</b>		$\alpha$ -Cyclodextrin
Succinamic acid		Tween 80
Glucoranimide		Tween 40
Alaninamide		<b>Esters</b>
		Mono-methylsuccinate
		Methylpyruvate

However, as many organisms are known to produce toxins designed to minimize competition (Curl and Truelove, 1986), plants that are able to coexist or the so-called co-occurring plants should be evaluated for their potential to enhance rhizoremediation of PAHs. Also, longer-term studies should be considered to corroborate these results.

The metabolic fingerprints of the PAH-contaminated soils were different from the metabolic fingerprints of the noncontaminated soils. This can be attributed to the change in microbial diversity due to the presence of PAH. The availability of hydrophobic pollutants has been reported to cause significant changes in soil microbial communities (Saxton and Atlas, 1977; Atlas *et al.*, 1991; Wünsche *et al.*, 1995). The metabolic fingerprint of the PAH-contaminated soil was more closely related to the contaminated monoplanted treatment with *Brachiaria serrata* and



**Table 6** Number of carbon sources that were not used by microorganisms in the different treatments ("total number of substrates used)

Treatment 0 (TO)* 54	Treatment 1 (T1) *80	Treatment 2 (T2) *75	Treatment 3 (T3) *76	Treatment 4 (T4) *60	Treatment 5 (T5) *77	Treatment 6 (T6) *67
<p><b>Carbohydrates</b> Adonitol, L-Arabinose, L-Fucose, Gentiobiose, <math>\alpha</math> -D-Lactose, <math>\alpha</math> -D-Lactose, D-Melibiose, <math>\beta</math> Methyl D-Glucoside <b>Carboxylic acids</b> Acetic acid, cis-Aconitic acid, D-Galactonic acid Lactone, D-Galacturonic acid, D-Glucosaminic acid, <math>\alpha</math> Hydroxybutyric acid, <math>\alpha</math> Keto butyric acid, Quinic acid, Succinic acid</p> <p><b>Amino acids</b> L-Alanine, L-Alanyl glycine, L-Asparagine, L-Aspartic acid, L-Glutamic acid, Glycyl L-Aspartic acid, Glycyl L-Glutamic acid, L-Ornithine, L-Threonine, D-Serine, <math>\gamma</math> Amino Butyric acid</p>	<p><b>Carbohydrates</b> N-Acetyl Galactosamine, D-Arabitol, Turanose, Xylitol <b>Carboxylic acids</b> cis-Aconitic acid, p-Hydroxy phenylacetic acid, <math>\alpha</math> -Keto valeric acid <b>Amino acids</b> L-Aspartic acid, L-Phenylalanine, L-Proline <b>Aromatic chemicals</b> Urocanic acid, Inosine, Thymidine <b>Amides</b> Glucuronamide <b>Brominated chemicals</b> Bromosuccinic acid</p>	<p><b>Carbohydrates</b> Adonitol, <math>\alpha</math> -D-Lactose, <math>\beta</math> Methyl D-Glucoside <b>Carboxylic acids</b> <math>\alpha</math> Hydroxybutyric acid, <math>\alpha</math> Keto butyric acid, Quinic acid, Succinic acid <b>Amino acids</b> L-Aspartic acid, L-Glutamic acid, Glycyl L-Aspartic acid, Glycyl L-Aspartic acid, L-Ornithine, L-Threonine, <math>\gamma</math> Amino Butyric acid <b>Phosphorylated chemicals</b> <math>\alpha</math>,D-Glucose-1-Phosphate, D-Glucose-6-Phosphate <b>Polymers</b> Dextrin, Tween 40 <b>Esters</b> Succinic acid monomethyl ester <b>Amines</b> Putrescine</p>	<p><b>Carbohydrates</b> Adonitol, <math>\beta</math> Methyl D Glucoside <b>Carboxylic acids</b> <math>\alpha</math> -Hydroxybutyric acid, <math>\alpha</math> Keto butyric acid, Quinic acid, Succinic acid <b>Amino acids</b> L-Aspartic acid, Glycyl L-Glutamic acid, L-Ornithine, L-Phenylalanine, L-Threonine, <math>\gamma</math> Amino Butyric acid <b>Phosphorylated chemicals</b> <math>\alpha</math>, D-Glucose-1-Phosphate, D-Glucose-6-Phosphate <b>Polymers</b> Dextrin, Tween 40 <b>Amides</b> Glucuronamide <b>Amines</b> Putrescine <b>Brominated chemicals</b> Bromosuccinic acid</p>	<p><b>Carbohydrates</b> N-Acetyl D-Glucosamine, Adonitol, L-Arabinose, D-Cellobiose, D-Fructose, L-Fucose, Gentiobiose, <math>\alpha</math> -D-Lactose, D-Melibiose, <math>\beta</math> Methyl D-Glucoside, Turanose <b>Carboxylic acids</b> Acetic acid, D-Galactonic acid Lactone, D-Galacturonic acid, D-Glucosaminic acid, <math>\alpha</math> Keto butyric acid, <math>\alpha</math> Hydroxybutyric acid, <math>\alpha</math> Keto glutaric acid, Malonic acid, Succinic acid <b>Amines</b> L-Glutamic acid, Glycyl L-Glutamic acid, <math>\gamma</math> Amino Butyric acid</p>	<p><b>Carbohydrates</b> N-Acetyl D-Glucosamine, L-Arabinose, Gentiobiose, <math>\alpha</math> -D-Lactose, Lactulose, Maltose, D-Melibiose, Xylitol <b>Carboxylic acids</b> Acetic acid, D-Galactonic acid Lactone, D-Galacturonic acid, Itaconic acid, <math>\alpha</math> Keto Glutaric acid, Malonic acid, Succinic acid <b>Polymers</b> Tween 80 <b>Esters</b> Succinic acid mono methyl ester <b>Brominated chemicals</b> Bromosuccinic acid</p>	<p><b>Carbohydrates</b> N-Acetyl D-Glucosamine, Adonitol, L-Arabinose, D-Cellobiose, D-Galactose, Gentiobiose, Lactulose, D-Melibiose, <math>\beta</math> Methyl D-Glucoside, Turanose, Xylitol <b>Carboxylic acids</b> D-Glucosaminic acid, <math>\alpha</math> Keto butyric acid, <math>\alpha</math> Keto Glutaric acid, p-Hydroxy phenylacetic acid <b>Amino acids</b> Glycyl L-Glutamic acid, L-Ornithine, L-Serine, <math>\gamma</math> Amino Butyric acid <b>Phosphorylated chemicals</b> D-L- <math>\alpha</math> -Glycerol Phosphate, D-Glucose-1-Phosphate, D-Glucose-6-Phosphate</p>

(Continued on next page)

**Table 6** Number of carbon sources that were not used by microorganisms in the different treatments ("total number of substrates used") (Continued)

Treatment 0 (T0)* 54	Treatment 1 (T1) *8	Treatment 2 (T2) *75	Treatment 3 (T3) *76	Treatment 4 (T4) *60	Treatment 5 (T5) *77	Treatment 6 (T6) *67
<b>Aromatic chemicals</b> Urocanic acid, Inosine, Uridine, Thymidine <b>Phosphorylated chemicals</b> D-Glucose-1-Phosphate, D-Glucose-6-Phosphate <b>Polymers</b> $\alpha$ -Cyclodextrin, Dextrin, Tween 40 <b>Esters</b> Succinic acid mono methyl ester <b>Amides</b> Glucuronamide <b>Alcohols</b> Glycerol <b>Amines</b> Putrescine,				<b>Aromatic chemicals</b> Uridine <b>Phosphorylated chemicals</b> D-Glucose-1-Phosphate, D-Glucose-6-Phosphate <b>Polymers</b> $\alpha$ -Cyclodextrin, Dextrin, Tween 40 <b>Esters</b> Pyruvic acid methyl ester, Succinic acid mono methyl ester <b>Alcohols</b> Glycerol		<b>Polymers</b> Dextrin, Tween 40, Tween 80 <b>Esters</b> Pyruvic acid methyl ester <b>Amines</b> Putrescine <b>Alcohols</b> Glycerol

contaminated multiplanted soil with *Brachiaria serrata* and *Eleusine corocana* than to other treatments. This can be attributed to the “similarities” in metabolic diversity of the contaminated treatments caused by the presence of the stressor (PAHs).

The functional diversities of the different treatments were further evaluated using hierarchical clustering. In a dendrogram, the contaminated soils (CS, CSBS, and CSBEC) were clustered together while CSEC was clustered with the uncontaminated soils. These results were perplexing as it was expected that CSEC, a planted:contaminated soil, was going to be clustered with other contaminated soil due to presence of the stressor, which can cause significant changes in soil microbial communities (Rathbone *et al.*, 1998). The number of Biolog substrates used by microorganisms in the planted treatments was higher compared to the nonplanted treatments. This can be attributed to the improved microbial competence brought by the presence of the plants.

In conclusion, plant-mediated dissipation of PAHs was enhanced in the multiplanted treatments compared to the mono-planted treatments. However, enhanced PAH dissipation in the multiplanted treatments compared to the mono-planted treatments did not correspond to high functional diversity, as revealed by the number of Biolog substrates used. The results suggest that multiplant rhizoremediation should be considered when evaluating remedial approaches for moderately contaminated soils. The results further strengthen the suggestion that “bioaugmentation” in moderately contaminated soils can be achieved by using multiplanted instead of monocultures to enhance the competence of the soil bacteria. However, further work is required to understand microbial diversity in multiplanted soil treatments, particularly as root exudates by one plant species may suppress or encourage the bacterial species “predominant” when each plant grows in isolation.

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