Journal of Environmental Management 155 (2015) 171–176

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Rhizoremediation of phenanthrene and pyrene contaminated soil using wheat

Esmaeil Shahsavari*, Eric M. Adetutu, Mohamed Taha, Andrew S. Ball

Centre for Environmental Sustainability and Remediation, School of Applied Sciences, RMIT University, Bundoora, Victoria 3083, Australia

ARTICLE INFO

Article history:
Received 11 November 2014
Received in revised form
12 March 2015
Accepted 17 March 2015
Available online 26 March 2015

Keywords:
Phytoremediation
Rhizoremediation
Plant rhizosphere
Polycyclic aromatic hydrocarbons
DGGE
MPN-qPCR

ABSTRACT

Rhizoremediation, the use of the plant rhizosphere and associated microorganisms represents a promising method for the clean up of soils contaminated with polycyclic aromatic hydrocarbons (PAHs) including phenanthrene and pyrene, two model PAHs. Although numerous studies have been published reporting the degradation of phenanthrene and pyrene, very few evaluate the microbial basis of the rhizoremediation process through the application of molecular tools. The aim of this study was to investigate the effect of wheat on the degradation of two model PAHs (alone or in combination) and also on soil bacterial, fungal and *nidA* gene (i.e. a key gene in the degradation of pyrene) communities. The addition of wheat plants led to a significant enhancement in the degradation of both phenanthrene and pyrene. In pyrene-contaminated soils, the degradation rate increased from 15% (65 mg/kg) and 18% (90 mg/kg) in unplanted soils to 65% (280 mg/kg) and 70% (350 mg/kg) in planted treatments while phenanthrene reduction was enhanced from 97% (394 mg/kg) and 87% (392 mg/kg) for unplanted soils to 100% (406 mg/kg) and 98% (441 mg/kg) in the presence of wheat. PCR-DGGE results showed that the plant root let to some changes in the bacterial and fungal communities; these variations did not reflect any change in hydrocarbon-degrading communities. However, plate counting, traditional MPN and MPN-qPCR of *nidA* gene revealed that the wheat rhizosphere led to an increase in the total microbial abundance including PAH degrading organisms and these increased activities resulted in enhanced degradation of phenanthrene and pyrene. This clearer insight into the mechanisms underpinning PAH degradation will enable better application of this environmentally friendly technique.

1. Introduction

Phytoremediation is defined as the use of plant and associated microorganisms to remove, or degrade contaminants in soil, water and air. This technique represents a cost effective, non-invasive eco-friendly method for the clean-up of environmental contamination (Arthur et al., 2005), that enjoys wide public acceptance. Petroleum-derived hydrocarbons (e.g. polycyclic aromatic hydrocarbons, PAHs) are a group of organic contaminants which have been successfully treated by plants through a phytoremediation technique termed rhizoremediation (Banks et al., 2003; Cheema et al., 2010; Gaskin and Bentham, 2010; Reilley et al., 1996; Shahsavari et al., 2013c). Generally, in rhizoremediation, the rhizosphere accelerates the degradation of petroleum hydrocarbons with its associated induced microorganisms. This technology can be useful for treating contaminants which cannot be taken up by plant tissue but can be degraded by microorganisms (Pilon-Smits, 2005).

Plant roots may enhance the degradation rate of petroleum hydrocarbons in different ways (Fig. 1). For example, plant roots stimulate microbial activity by releasing nutrients, exudates and oxygen into the soil (Macek et al., 2000). It is estimated that 40–90% of the stored carbon in the root of an annual plant is released into the soil via rhizodeposition (Olson et al., 2004). As a result, the density of microorganisms increase up to 10–1000 fold in planted soil relative to unplanted soil. In addition, released carbon, exudates and oxygen in the soil leads to enhanced soil aeration and also improved soil quality. In terms of PAH degradation, plants may induce the microbial genes responsible for the early stage of PAH degradation. One compound, salicylate plays an important role in plant signaling and has also been reported as an inducer of the *nah* gene (the gene is involved in naphthalene degradation) (Yen et al., 1988).

* Corresponding author.
E-mail address: esmaeil.shahsavari@rmit.edu.au (E. Shahsavari).

http://dx.doi.org/10.1016/j.jenvman.2015.03.027
0301-4797/

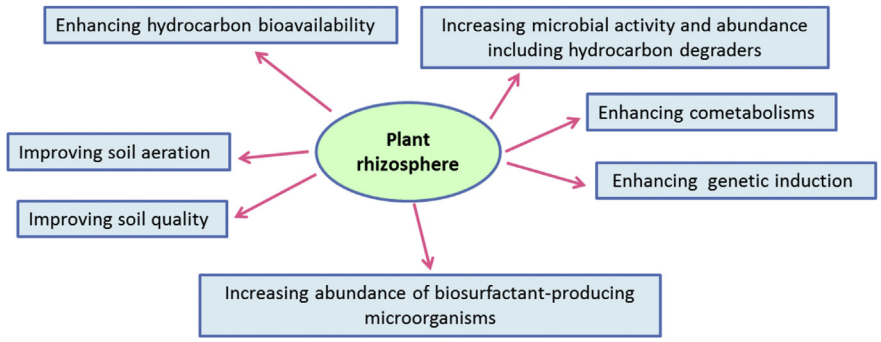

Fig. 1. Proposed beneficial effects of plant rhizosphere on the degradation of petroleum hydrocarbons (Olson et al., 2004).

Among petroleum hydrocarbon compounds, PAHs are toxic, mutagenic and carcinogenic. In addition, PAHs are recalcitrant and persist in soils for a long, for example, the half-life of benz[a] anthracene in soil is 6,250 d (Mougin, 2002). Consequently, the USEPA has categorized 16 dangerous PAHs, including phenanthrene and pyrene as priority contaminants. Phenanthrene and pyrene are used as model compounds for 3-ring and 4-ring PAHs, respectively, in many phytoremediation studies (Cheema et al., 2010, 2009; Chen and Banks, 2004; Chen et al., 2003; D'Orazio et al., 2013; Lee et al., 2008; Liste and Alexander, 2000; Lu et al., 2014; Wang et al., 2014; Wei and Pan, 2010). Lee et al. (2008) investigated the degradation of phenanthrene and pyrene by four native Korean plant species (*Panicum bisulcatum*, *Echinogalus crus-galli*, *Astragalus membranaceus*, and *Aeschynomene indica*) during an 80-day experiment. The results showed that >99% and between 77% and 94% of phenanthrene and pyrene, respectively were degraded in planted soil, while the degradation of these two PAHs in control soil was 99% and 69%. In another study, D'Orazio et al. (2013) investigated the potential effect of 3 plant species, namely: alfalfa, oilseed rape and ryegrass on the degradation of pyrene in a greenhouse-based study. The authors reported that pyrene concentration was reduced to 32%, 30% and 28% in soils planted with alfalfa, oilseed rape and ryegrass, respectively, while it was reduced only to 18% in the control after 90 d of incubation.

In spite of the fact that many reports are available in the literature regarding the phytoremediation of phenanthrene and pyrene as PAH model compounds, only a few of these reports assessed the soil microbial community using molecular tools. Chen and Banks (2004) used polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE) to assess the bacterial community in a pyrene-spiked soil planted with tall fescue. The authors were not able to see any changes in bacterial community in spite of the fact that the presence of the tall fescue plants significantly enhanced the degradation of pyrene relative to unplanted soil. In addition, the authors did not assess the fungal community.

It is well known that not all plant species are suitable for phytoremediation (Shahsavari et al., 2013c). In our previous study (Shahsavari et al., 2013c), 11 plant species were assessed in order to determine which plants could be used for phytoremediation projects in a hydrocarbon contaminated soil. Our results showed that wheat was one of the plants that demonstrated a high potential for use in the degradation of diesel/oil engine and crude oil sludge among the 11 tested plants. Therefore, the objective of this study was to further investigate the effect of wheat plants on the degradation of model PAHs (phenanthrene and pyrene) alone or in combination. In addition, both molecular tools (PCR-DGGE and Most probable number-qPCR; MPN-qPCR) and traditional methods were used to investigate the effects of rhizoremediation on the soil contaminated with phenanthrene and pyrene.

2. Materials and methods

2.1. Experimental design

An uncontaminated sandy loam (clean) soil from South Australia was used in pots for this study. Physical and chemical characteristics of the soil have been described previously (Shahsavari et al., 2013b).

The experiment was carried out in a greenhouse at 25–32 °C. The contamination of the soil samples was performed as described previously (Shahsavari et al., 2013a). The initial concentration of phenanthrene and pyrene was 406 ± 48 and 430 ± 60 mg/kg respectively when spiked individually, while when added in combination, the concentration was found to be 450 ± 60 and 500 ± 70 mg/kg for phenanthrene and pyrene respectively.

Wheat seeds (n = 5) were sown into each pot containing 1 kg of either contaminated or uncontaminated soil. The number of seedlings was thinned to three when the seedlings length was ~3–4 cm. In total, seven treatments and controls pots (each in triplicate) were set up (Table 1). All pots were watered as appropriate so that a water holding capacity of 70% was maintained throughout the experiment. After 90 d, shoots and roots were harvested, with the roots being shaken by hand to remove loose unwanted soil. The attached rhizosphere soil and also control and clean soils were collected for PAH and microbial analyses.

2.2. Measurement of plant parameters

The shoots and roots were harvested, washed with tap water and dried gently with filter paper on a bench in a greenhouse. Shoot lengths were measured after 30, 60 and 90 d while root lengths were measured after 90 d. All samples were oven-dried at 75 °C for 72 h. The dry weights of shoots and roots were measured gravimetrically and expressed as root/shoot ratio (dry mass of roots/dry mass of shoots).

2.3. PAH analysis

The soil samples were sent to an external analytical laboratory (Flinders Advanced Analytical Laboratory, Flinders University, Australia) for the analysis of PAH concentration. The phenanthrene and pyrene concentrations of the soil samples were measured with Gas Chromatography as described by Makadia et al. (2011).

2.4. Microbial enumerations of soil samples

Enumeration of total viable bacteria, actinobacteria and fungi in soils was performed using plate counting methods, described elsewhere (Peng et al., 2009). In addition, a most probable number

Table 1
Treatments used throughout this study.

Treatments	Symbol
Uncontaminated soil + wheat plant	CS
Uncontaminated soil + phenanthrene (control)	CA
Uncontaminated soil + phenanthrene + wheat plant	PA
Uncontaminated soil + pyrene (control)	CB
Uncontaminated soil + pyrene + wheat plant	PB
Uncontaminated soil + phenanthrene + pyrene (control)	CC
Uncontaminated soil + phenanthrene + pyrene + wheat plant	PC

(MPN) method described by Gaskin and Bentham (2005) was used to enumerate the PAH degraders.

2.5. Microbial community structure

Extraction of total DNA and RNA was carried out from soils according to the method of Griffiths et al. (2000). The cDNA was prepared using the Reverse Transcription System (Promega, Australia) according to the manufacturer's protocol as described previously (Shahsavari et al., 2013b). The DNA and cDNA were stored at −20 °C until further analyses.

The total (16S rDNA) and active (16S rRNA (cDNA)) soil bacterial community was amplified using 341F GC and 518R primers according to the method of Muyzer et al. (1993). A nested PCR method was used to evaluate the fungal community by using ITS1, IT1F GC, ITS2 and ITS4 primers (ITS region based primers) as described by Sheppard et al. (2011). The PAH degrading community (based on the detection of *nidA* genes) was analyzed using *nidA* based primers (Sho et al., 2004) using a semi-nested PCR as described previously by Makadia et al. (2011); for the *nidA* gene based analysis, the GC clamp was attached to the forward primer. DGGE analysis was carried out on the PCR amplicons using a D-Code System (BioRad). PCR products were loaded onto a 9% urea-formamide denaturant gradient polyacrylamide gel with a denaturing gradient of 45–65% for bacteria and 42–52% for fungi and *nidA* gene communities and run for 20 h at 60 °C and 60 V. The DGGE gels were silver stained (Girvan et al., 2003) or SYBR gold. Dominant and unique bands on the bacterial and fungal DGGE gels were excised and sequenced as described earlier (Adetutu et al., 2011). Images of DGGE gels were scanned and subjected to analysis with Phoretix 1D software to create a dendrogram.

2.6. Gene copy number of nidA genes using MPN-qPCR

Enumeration of *nidA* gene (a key gene in degradation of pyrene and most likely phenanthrene) copy numbers was performed using MPN-qPCR, based on methods described by Pérez-de-Mora et al. (2010). Serial dilutions of prepared DNA of different treatments (control and planted soils) were used for PCR using the *nidA* primers (Sho et al., 2004).

2.7. Data analysis

Analysis of variance (ANOVA) was carried out by using IBM SPSS software (version 21) for all data. Mean values were separated using the Least Significant Difference (LSD test; $P = 0.05$), if the F-value was significant. The standard error (SE) was used where required. In the present study some mean values from control soils were presented in Shahsavari et al. (2013a). In addition, the principal component analysis (PCA) on microbial DGGE profiles was also carried out with IBM SPSS software (version 21).

3. Results and discussion

3.1. Effect of PAHs on wheat growth

Seedling emergence of wheat plants was 100% in both clean and PAH-contaminated soils. This finding further validates our previous results that showed that diesel or crude oil sludge did not have any adverse effects on wheat emergence rate (Shahsavari et al., 2013c).

To assess the effects of PAHs on shoot growth of wheat, the shoot length of each treatment was measured after 30, 60, or 90 d. The results showed that addition of phenanthrene and pyrene alone or in combination led to a significant reduction in shoot length in contaminated soils relative to clean soil (Fig. 2a). These differences in clean and contaminated soils were more evident after 30 d of experiments. For example, at the end of the

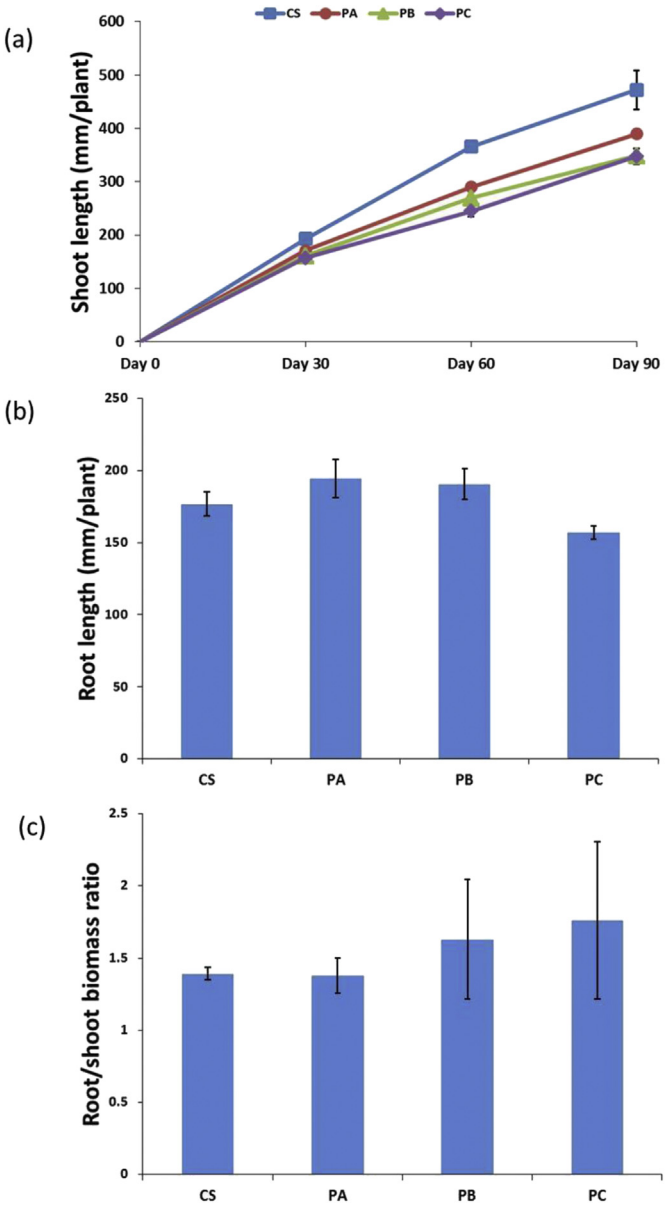

Fig. 2. Effects of phenanthrene and pyrene on wheat shoot length (a), root length (b) and dry shoot/root biomass ratio (c) after 90 d of experiment. CS: Uncontaminated soil + wheat plant, PA: Uncontaminated soil + phenanthrene + wheat plant, PB: Uncontaminated soil + pyrene + wheat plant, PC: Uncontaminated soil + phenanthrene + pyrene + wheat plant.

experiment, the shoot length in PA (soil + phenanthrene + wheat plant), PB (soil + pyrene + wheat plant) and PC (soil + phenanthrene + pyrene + wheat plant) treatments was 389.8, 348.3 and 347.2 mm/plant, respectively, while the shoot length in clean soil was 471.9 mm/plant. The highest reduction of shoot growth was 26%, observed in PC treatments (Fig. 2a). In contrast, there were no significant differences between clean soil and contaminated soils in terms of root length (Fig. 2b). The dry root/shoot biomass ratio and root length were measured at the end of the experiments. The dry root/shoot biomass ratio was higher in treatments PB (uncontaminated soil + pyrene + wheat plant) and PC (Uncontaminated soil + phenanthrene + pyrene + wheat plant) compared with PA (uncontaminated soil + phenanthrene + wheat plant) and CS (CS: uncontaminated soil + wheat plant); however, there were no significant differences observed between them (Fig. 2c).

Although the addition of PAHs in this study decreased shoot growth, the exposure was not lethal and the aboveground parts of the wheat plant could still provide photosynthetic products to the roots. Peng et al. (2009) reported that the presence of 10,000 mg/kg petroleum hydrocarbons (TPH) in soil decreased shoot growth of four o'clock flower plants by 23.6% compared to its unplanted control soil. D'Orazio et al. (2013) showed that soil contaminated with 100 mg/kg pyrene led to a decrease in the shoot growth of alfalfa and oilseed rape while this level of pyrene did not affect the shoot growth in ryegrass. In this study, in contrast, both root length and dry biomass ratio in plants growth in contaminated soils were not significantly different to those values obtained for plants from control soils, confirming that wheat roots may still play an important role in the degradation of phenanthrene and pyrene.

3.2. Phenanthrene and pyrene concentration in contaminated soils

The effects of wheat plants on the degradation of phenanthrene and pyrene are shown in Table 2. In all treatments, the presence of wheat had a positive impact on the degradation of phenanthrene and pyrene. In pyrene-contaminated soils planted with wheat, the degradation rate was 65% (280 mg/kg) in PB and 70% (350 mg/kg) in PC treatments respectively compared to 15% (65 mg/kg) and 18% (90 mg/kg) in corresponding control soils (Table 2). The % reduction of phenanthrene in planted treatments when used alone or in combination was 100% (406 mg/kg) and 98% (441 mg/kg), respectively, compared with 97% (394 mg/kg) and 87% (392 mg/kg) for unplanted soils.

These results are supported by numerous studies reported in the literature with regard to these two types of model PAHs (Cheema et al., 2010, 2009; Chen and Banks, 2004; D'Orazio et al., 2013; Liste and Alexander, 2000; Xu et al., 2006). Liste and Alexander (2000) evaluated the ability of nine plant species to increase the degradation of pyrene in soil. The authors reported that pyrene reduction in planted soils was 74% compared to 40% from unplanted control soil after 8 weeks of experiment.

In another study, Cheema et al. (2009) showed that the presence of tall fescue plants led to enhanced degradation of phenanthrene, with an average increase in utilization of 1.88–3.19% and pyrene with an average increase in utilization of 8.85–20.69% compared to corresponding control soils.

3.3. Effects of wheat plant on microbial activities of PAH-contaminated soil

The results of plate counting showed that the number of bacteria was significantly higher in planted treatments relative to the corresponding control soils (Fig. 3). However, there were no significant differences observed among the plant treatments. The bacterial population in PA, PB and PC treatments was 8.02, 7.95 and 7.99 $\log_{10}$ CFU/g dry soil compared to 6.29, 6.74 and 7.15 $\log_{10}$ CFU/g dry soil in corresponding controls (CA, CB and CC treatments). A similar trend was also observed in actinobacterial and fungal populations confirming that the presence of wheat plants led to a significant increase in the numbers of microorganisms in contaminated soils, with the exception of actinobacterial numbers in PA treatment.

In terms of PAH degraders, in the PA treatment the number of PAH degraders was not significantly different from its control (CA). In contrast, the number of PAH degraders in PB and PC treatments increased significantly from 4.51 to 4.62 $\log_{10}$ CFU/g dry soil in the control soil (BC and CC) to 5.51 and 6.02 $\log_{10}$ CFU/g dry soil respectively (Fig. 3).

Similar results were also reported by Cheema et al. (2009) which showed that the bacterial population of soils planted with tall fescue (6.33×10^7 to 11.85×10^7 CFU/g dry soil) were significantly higher than in unplanted control soils (3.01×10^7 to 5.69×10^7 CFU/g dry soil). The authors also reported that the numbers of fungi in planted soils were 1.5–2 fold higher than in unplanted soils. These increased activities may be as a result of root exudates and oxygen released from plant roots in to the soil. Plants roots release substantial amounts of organic compounds such as amino acids, organic acids, sugars, enzymes and polymeric carbohydrates into the soil which provide a rich environment for microbial growth (Olson et al., 2004).

Table 2
Reduction rate of phenanthrene and pyrene in wheat planted and control soils after 90 d of experiment.

	Treatments					
	CA	PA	CB	PB	CC	PC
Phenanthrene (%)	97[b*]	100[a]	–	–	87[c]	98[a]
Pyrene (%)	–	–	15[b]	65[a]	18[b]	70[a]

CA: Uncontaminated soil + phenanthrene (control), PA: Uncontaminated soil + phenanthrene + wheat plant, CB: Uncontaminated soil + pyrene (control), PB: Uncontaminated soil + pyrene + wheat plant, CC: Uncontaminated soil + phenanthrene + pyrene (control), PC: Uncontaminated soil + phenanthrene + pyrene + wheat plant.
*Means with the different letter are significantly different in each row at $P \leq 0.05$ using LSD test.
–Not detected.

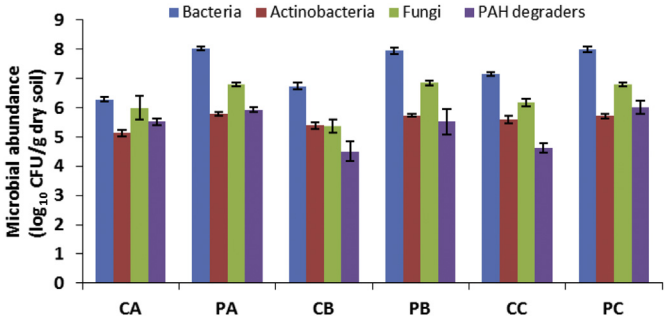

Fig. 3. Microbial population of planted and control soils contaminated with phenanthrene and pyrene after 90 d of experiment. CA: Uncontaminated soil + phenanthrene (control), PA: Uncontaminated soil + phenanthrene + wheat plant, CB: Uncontaminated soil + pyrene (control), PB: Uncontaminated soil + pyrene + wheat plant, CC: Uncontaminated soil + phenanthrene + pyrene (control), PC: Uncontaminated soil + phenanthrene + pyrene + wheat plant.

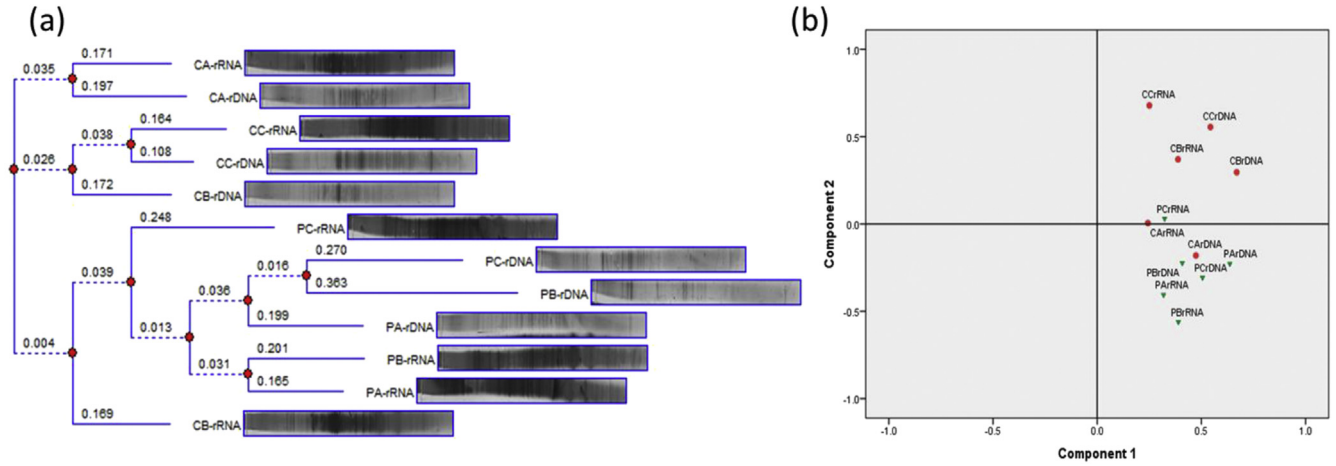

Fig. 4. Dendrogram (a) and PCA analysis (b) of total (16S rDNA) and active bacterial (16S rRNA) communities in planted and control soils. CA: Uncontaminated soil + phenanthrene (control), PA: Uncontaminated soil + phenanthrene + wheat plant, CB: Uncontaminated soil + pyrene (control), PB: Uncontaminated soil + pyrene + wheat plant, CC: Uncontaminated soil + phenanthrene + pyrene (control), PC: Uncontaminated soil + phenanthrene + pyrene + wheat plant.

3.4. *Effects of wheat plants on microbial communities of contaminated soil and nidA gene abundance*

Cluster analysis following DGGE was used to assess the effects of plants on bacterial (total and active bacteria) and fungal soil communities. The results showed that in control soils, both total (16s rDNA) and active (16S rRNA) bacteria clustered differently from planted treatments (no specific trends were observed between rDNA and rRNA) (Fig. 4a). PCA analysis showed that both total and active bacterial communities in pyrene-contaminated soil controls grouped differently from other treatments, confirming that wheat plants resulted in some shifts in bacterial communities in pyrene treatments (Fig. 4b).

In terms of fungi, cluster analysis of DGGE profiles and PCA analysis revealed that the presence of a plant rhizosphere led to some shifts in the fungal community. The contaminated soil plants with wheat grouped differently from contaminated soil without wheat plants (Fig. 5a and 5b).

In terms of the *nidA* gene community, results showed that the addition of phenanthrene and pyrene or wheat plants did not change the *nidA* gene community, with DGGE profiles remaining the same irrespective of treatment. However, in the pyrene treatments (planted and control soils) only one extra band was observed (data not shown).

Sequencing of the bands of interest in bacterial and fungal communities showed that some of these bacteria and fungi in the DGGE profiles represented known hydrocarbon degraders. However, most of these proposed hydrocarbon degraders were also detected in control soil suggesting that the hydrocarbonoclastic microorganisms are members of the normal soil microflora. MPN-qPCR showed that *nidA* gene copy numbers in PAH-contaminated soil planted with wheat were much higher than the corresponding controls (data not shown). The gene copies in planted treatments showed 3.5-, 5.6- and 3-fold increase. The *nidA* gene codes the large subunit of pyrene dioxygenase which catalyzes the initial step of PAH metabolism. In addition, *nidA* may also be involved in phenanthrene degradation (Stingley et al., 2004). In this study, wheat plants led to an increase in the *nidA* gene copy number in contaminated soil. This result suggests that this gene can be used as biomarker for monitoring phytoremediation of phenanthrene and pyrene. The *nidA* gene has also been used as a biomarker in PAH-contaminated soils (DeBruyn et al., 2007; Peng et al., 2010). Peng et al. (2010) showed that *nidA* gene copy numbers could be used to predict pyrene contamination and its degradation activity in soils.

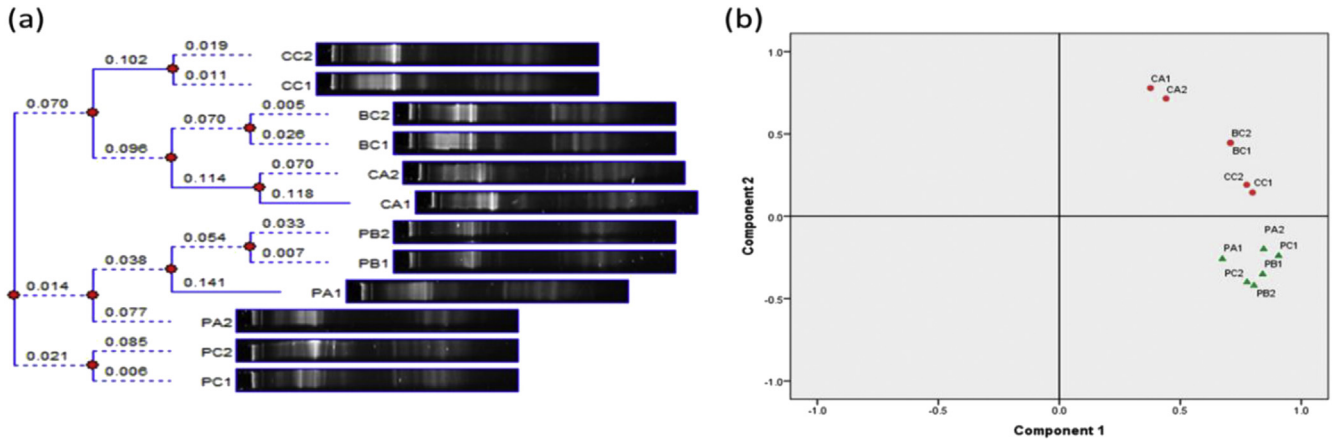

Fig. 5. Dendrogram (a) and PCA analysis (b) of fungal community in planted and control soils. CA: Uncontaminated soil + phenanthrene (control), PA: Uncontaminated soil + phenanthrene + wheat plant, CB: Uncontaminated soil + pyrene (control), PB: Uncontaminated soil + pyrene + wheat plant, CC: Uncontaminated soil + phenanthrene + pyrene (control), PC: Uncontaminated soil + phenanthrene + pyrene + wheat plant.

4. Conclusion

The results from this study revealed that phytoremediation using wheat can be an effective treatment for the degradation of phenanthrene and pyrene. The presence of wheat roots led to an increase in microbial activity together with some changes in both bacterial and fungal communities as assessed by PCR-DGGE. However, these changes did not reflect any shift in PAH-degrading community in plant treatments, confirming the soil used in this experiment already contained PAHs degraders, although these hydrocarbon degraders were not active due to the poor soil conditions (e.g. insufficient nutrient). The MPN and *nidA* gene copy numbers showed that the presence of wheat roots increased the abundance of PAHs degraders. Therefore, planting the soils with wheat resulted in an increase in PAHs degradation, presumably through the addition of root exudes and oxygen into the soils.

References

Adetutu, E.M., Thorpe, K., Bourne, S., Cao, X., Shahsavari, E., Kirby, G., Ball, A.S., 2011. Phylogenetic diversity of fungal communities in areas accessible and not accessible to tourists in Naracoorte caves. Mycologia 103, 959–968.

Arthur, E.L., Rice, P.J., Rice, P.J., Anderson, T.A., Baladi, S.M., Henderson, K.L., Coats, J.R., 2005. Phytoremediation—an overview. Crit. Rev. Plant Sci. 24, 109–122.

Banks, M., Kulakow, P., Schwab, A., Chen, Z., Rathbone, K., 2003. Degradation of crude oil in the rhizosphere of *Sorghum bicolor*. Int. J. Phytoremediation 5, 225–234.

Cheema, S.A., Imran Khan, M., Shen, C., Tang, X., Farooq, M., Chen, L., Zhang, C., Chen, Y., 2010. Degradation of phenanthrene and pyrene in spiked soils by single and combined plants cultivation. J. Hazard. Mater. 177, 384–389.

Cheema, S.A., Khan, M.I., Tang, X., Zhang, C., Shen, C., Malik, Z., Ali, S., Yang, J., Shen, K., Chen, X., Chen, Y., 2009. Enhancement of phenanthrene and pyrene degradation in rhizosphere of tall fescue (*Festuca arundinacea*). J. Hazard. Mater. 166, 1226–1231.

Chen, Y.-C., Banks, M.K., 2004. Bacterial community evaluation during establishment of tall fescue (*Festuca arundinacea*) in soil contaminated with pyrene. Int. J. Phytoremediation 6, 227–238.

Chen, Y.-C., Banks, M.K., Schwab, A.P., 2003. Pyrene degradation in the rhizosphere of tall fescue (*Festuca arundinacea*) and switchgrass (*Panicum virgatum* L.). Environ. Sci. Technol. 37, 5778–5782.

D'Orazio, V., Ghanem, A., Senesi, N., 2013. Phytoremediation of pyrene contaminated soils by different plant species. Clean. Soil Air Water 41, 377–382.

DeBruyn, J.M., Chewning, C.S., Sayler, G.S., 2007. Comparative quantitative prevalence of *Mycobacteria* and functionally abundant *nidA*, *nahAc*, and *nagAc* dioxygenase genes in coal tar contaminated sediments. Environ. Sci. Technol. 41, 5426–5432.

Gaskin, S., Bentham, R., 2005. Comparison of enrichment methods for the isolation of pyrene-degrading bacteria. Int. Biodeterior. Biodegrad. 56, 80–85.

Gaskin, S.E., Bentham, R.H., 2010. Rhizoremediation of hydrocarbon contaminated soil using Australian native grasses. Sci. Total Environ. 408, 3683–3688.

Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M., Ball, A.S., 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Appl. Environ. Microbiol. 69, 1800–1809.

Griffiths, R.I., Whiteley, A.S., O'Donnell, A.G., Bailey, M.J., 2000. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA-and rRNA-based microbial community composition. Appl. Environ. Microbiol. 66, 5488–5491.

Lee, S.-H., Lee, W.-S., Lee, C.-H., Kim, J.-G., 2008. Degradation of phenanthrene and pyrene in rhizosphere of grasses and legumes. J. Hazard. Mater. 153, 892–898.

Liste, H.-H., Alexander, M., 2000. Plant-promoted pyrene degradation in soil. Chemosphere 40, 7–10.

Lu, M., Zhang, Z.-Z., Wang, J.-X., Zhang, M., Xu, Y.-X., Wu, X.-J., 2014. Interaction of heavy metals and pyrene on their fates in soil and tall fescue (*Festuca arundinacea*). Environ. Sci. Technol. 48, 1158–1165.

Macek, T., Mackova, M., Káš, J., 2000. Exploitation of plants for the removal of organics in environmental remediation. Biotechnol. Adv. 18, 23–34.

Makadia, T.H., Adetutu, E.M., Simons, K.L., Jardine, D., Sheppard, P.J., Ball, A.S., 2011. Re-use of remediated soils for the bioremediation of waste oil sludge. J. Environ. Manag. 92, 866–871.

Mougin, C., 2002. Bioremediation and phytoremediation of industrial PAH-polluted soils. Polycycl. Aromat. Compd. 22, 1011–1043.

Muyzer, G., De Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59, 695–700.

Olson, P.E., Reardon, K.F., Pilon-Smits, E.A.H., 2004. Ecology of rhizosphere bioremediation. In: McCutcheon, S.C., Schnoor, J.L. (Eds.), Phytoremediation: Transformation and Control of Contaminants. Wiley-Interscience, Inc., Hoboken, New Jersey, pp. 317–353.

Peng, J.-J., Cai, C., Qiao, M., Li, H., Zhu, Y.-G., 2010. Dynamic changes in functional gene copy numbers and microbial communities during degradation of pyrene in soils. Environ. Pollut. 158, 2872–2879.

Peng, S., Zhou, Q., Cai, Z., Zhang, Z., 2009. Phytoremediation of petroleum contaminated soils by *Mirabilis jalapa* L. in a greenhouse plot experiment. J. Hazard. Mater. 168, 1490–1496.

Pérez-de-Mora, A., Schulz, S., Schloter, M., 2010. MPN- and real-time-based PCR methods for the quantification of alkane monooxygenase homologous genes (alkB) in environmental samples. In: Cummings, S.P. (Ed.), Bioremediation. Humana Press, pp. 59–68.

Pilon-Smits, E., 2005. Phytoremediation. Annu. Rev. Plant Biol. 56, 15–39.

Reilley, K., Banks, M., Schwab, A., 1996. Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. J. Environ. Qual. 25, 212–219.

Shahsavari, E., Adetutu, E.M., Anderson, P.A., Ball, A.S., 2013a. Necrophytoremediation of phenanthrene and pyrene in contaminated soil. J. Environ. Manag. 122, 105–112.

Shahsavari, E., Adetutu, E.M., Anderson, P.A., Ball, A.S., 2013b. Plant residues - a low cost, effective bioremediation treatment for petrogenic hydrocarbon-contaminated soil. Sci. Total Environ. 443, 766–774.

Shahsavari, E., Adetutu, E.M., Anderson, P.A., Ball, A.S., 2013c. Tolerance of selected plant species to petrogenic hydrocarbons and effect of plant rhizosphere on the microbial removal of hydrocarbons in contaminated soil. Water Air & Soil Pollut. 224, 1–14.

Sheppard, P.J., Adetutu, E.M., Makadia, T.H., Ball, A.S., 2011. Microbial community and ecotoxicity analysis of bioremediated, weathered hydrocarbon-contaminated soil. Soil Res. 49, 261–269.

Sho, M., Hamel, C., Greer, C.W., 2004. Two distinct gene clusters encode pyrene degradation in *Mycobacterium* sp. strain S65. FEMS Microbiol. Ecol. 48, 209–220.

Stingley, R.L., Khan, A.A., Cerniglia, C.E., 2004. Molecular characterization of a phenanthrene degradation pathway in *Mycobacterium vanbaalenii* PYR-1. Biochem. Biophysical Res. Commun. 322, 133–146.

Wang, W., Zhang, X., Huang, J., Yan, C., Zhang, Q., Lu, H., Liu, J., 2014. Interactive effects of cadmium and pyrene on contaminant removal from co-contaminated sediment planted with mangrove *Kandelia obovata* (S., L.) Yong seedlings. Mar. Pollut. Bull. 84, 306–313.

Wei, S., Pan, S., 2010. Phytoremediation for soils contaminated by phenanthrene and pyrene with multiple plant species. J. Soils Sediments 10, 886–894.

Xu, S.Y., Chen, Y.X., Wu, W.X., Wang, K.X., Lin, Q., Liang, X.Q., 2006. Enhanced dissipation of phenanthrene and pyrene in spiked soils by combined plants cultivation. Sci. Total Environ. 363, 206–215.

Yen, K.-M., Serdar, C.M., Gunsalus, I.C., 1988. Genetics of naphthalene catabolism in pseudomonads. Crit. Rev. Microbiol. 15, 247–268.