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Characterization of successional changes in bacterial community composition during bioremediation of used motor oil-contaminated soil in a boreal climate

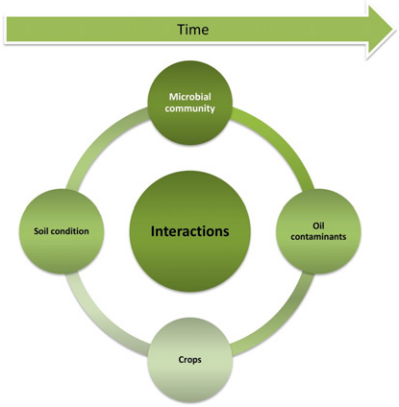
Lijuan Yan [a,*], Hanna Sinkko [b], Petri Penttinen [a], Kristina Lindström [a]

[a] Department of Environmental Sciences, PO Box 65 (Viikinkaari 2a), 00014, University of Helsinki, Finland
[b] Department of Food and Environmental Sciences, PO Box 56 (Latokartanonkaari 11), 00014, University of Helsinki, Finland

HIGHLIGHTS

- The impact of used motor on soil bacteria was monitored for four years.
- Bacterial community went through an oil contamination level-dependent succession.
- At the early stage oil effect on bacterial community composition was abrupt.
- The oil effect did not last as long as the oil in soil.
- Crops had no detectable effect on bacterial community composition.

GRAPHICAL ABSTRACT



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ABSTRACT

The widespread use of motor oil makes it a notable risk factor to cause scattered contamination in soil. The monitoring of microbial community dynamics can serve as a comprehensive tool to assess the ecological impact of contaminants and their disappearance in the ecosystem. Hence, a field study was conducted to monitor the ecological impact of used motor oil under different perennial cropping systems (fodder galega, brome grass, galega-brome grass mixture and bare fallow) in a boreal climate zone. Length heterogeneity PCR characterized a successional pattern in bacterial community following oil contamination over a four-year bioremediation period. Soil pH and electrical conductivity were associated with the shifts in bacterial community composition. Crops had no detectable effect on bacterial community composition or complexity. However, the legume fodder galega increased soil microbial biomass, expressed as soil total DNA. Oil contamination induced an abrupt change in bacterial community composition at the early stage, yet the effect did not last as long as the oil in soil. The successional variation in bacterial community composition can serve as a sensitive ecological indicator of oil contamination and remediation in situ.

* Corresponding author at: Department of Environmental Sciences, PO Box 65, 00014, University of Helsinki, Finland.
E-mail address: lijuan.yan@helsinki.fi (L. Yan).

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1. Introduction

Petroleum hydrocarbons (PHCs) originating from crude oil or refined petroleum products are detrimental to environmental health as soil contaminants. Used motor oil or crankcase oil is lubricating oil that is removed from the crankcase of internal combustion engines of vehicles (Irwin et al., 1997). The widespread handling of small volumes of used motor oil by enterprises, farms and private persons makes it a notable risk factor to cause scattered contamination. Besides physical removal (leaching and volatilization), PHCs are subjected to biodegradation, the metabolic ability of microorganisms to transform or mineralize organic contaminants to less harmful, non-hazardous substances (Margesin and Schinner, 1997; Margesin and Schinner, 2001; Namkoong et al., 2002; Chaîneau et al., 2003). Hydrocarbon fractions differ in their susceptibility to microbial attack (Leahy and Colwell, 1990). In used motor oil, the concentrations of long-chain aliphatics, benzene-, and naphthalene-based compounds, polycyclic aromatic hydrocarbons (PAHs) and heavy metals are high; once released, these carcinogenic compounds can result in long lasting contamination due to their high resistance to microbial degradation (Irwin et al., 1997; Dominguez-Rosado et al., 2004).

Nitrogen is often a limiting factor in biodegradation of hydrocarbon-contaminated soils. Leguminous plants that are resistant to hydrocarbon pollutants assist bioremediation of oil-polluted sites effectively and sustainably as substitutes of N-fertilizers (Dominguez-Rosado et al., 2004; Kamath et al., 2004; Chiapusio et al., 2007). The perennial legume fodder galega (*Galega orientalis*) and smooth brome grass (*Bromus inermis*) are both suitable to grow in a boreal climate and have great potential to enhance bioremediation of oil-contaminated soil in microcosm and mesocosm studies (Suominen et al., 2000; Kulakow et al., 2000; Lindstrom et al., 2003; Kaksonen et al., 2006; Muratova et al., 2008; Jasinskas et al., 2008; Kryževičienė et al., 2008; Mikkonen et al., 2011a). Further assistance to the bioremediation process may be provided by plant growth promoting bacteria (PGPB) that have potential to mitigate plant stress response and increase the bioavailability of soil contaminants, therefore enhancing the degradation of contaminants (Gurska et al., 2009; Hong et al., 2011; Pajuelo et al., 2011; Bhattacharyya and Jha, 2012).

Effectiveness and completeness are ultimate goals in a successful remediation project (White et al., 1998). Complete removal of contaminants in the environment is not always easy to achieve. White et al. (1998) proposed an ecologically based test of "how clean is clean" using assessment of microbial community dynamics as a comprehensive tool to estimate contaminant disappearance. Hence, understanding the successional dynamics of bacterial communities on contaminated sites is an important aspect of risk assessment needed for the planning of following remediation actions. Due to the operational simplicity and high reproducibility in analyzing large sample series, length heterogeneity analysis of polymerase chain reaction products (LH-PCR, Suzuki et al., 1998) was widely used to monitor the succession of microbial communities in response to oil pollution (Mills et al., 2003; Mills et al., 2006; Mikkonen et al., 2011b; Mikkonen et al., 2012). The possibility to compare the sizes of the amplicons against 16S rRNA gene sequences in silico enables preliminary identification of bacterial groups in the community (Mills et al., 2003; Tiirola et al., 2003).

To date, bacterial community succession in used motor oil-polluted soil in a boreal climate zone has received little experimental attention. The studies on bacterial community succession in oil-polluted vegetated soil have been limited to short-term microcosm and mesocosm experiments (Mikkonen et al., 2011b; Mukherjee et al., 2013; Simarro et al., 2013). The successional patterns of soil microbial community following oil contamination in a boreal field are plausibly different from those in short-term controlled conditions. Hence, a systematic field bioremediation study was established with the main aim to monitor the impact of used motor oil, different perennial cropping systems (fodder galega, brome grass, galega–brome grass mixture and bare fallow), plant growth promoting bacteria and soil parameters on bacterial community composition over a four-year period (2009–2012) in a boreal region, using LH-PCR microbial community fingerprinting analysis.

2. Materials and methods

2.1. Experimental design, samplings and chemical analysis of soil

The multi-year bioremediation field experiment was established in a split-plot design at Viikki experimental farm, Helsinki, Finland (60°14′N, 25°01′E, 8 m AMSL). Crop treatments of monocultures of brome grass and fodder galega, their mixture and bare fallow were the main plots in four replicated blocks. Used motor treatments (oil +/−) and plant growth promoting bacteria treatments (PGPB +/−) were the sub-plot factors. About 6 kg of used motor oil (Teboil Lubricants Classic Mineral Motor oil, SAE 10W-30, API SF/CD, Finland) was mixed with 10 kg of coarse sand (0.5–1.2 mm), spread and spiked onto the top 20 cm of each designated-to-be oil-contaminated plot with a rotary tiller on 17 June 2009, making the target contamination approximately to 7000 ppm (7 g kg^{-1} dry soil). The non-contaminated control plots received pure sand on the top 20 cm soil. Before sowing, seeds of *G. orientalis* cv. 'Gale' (Naturcom Oy, Ruukki, Finland) were all inoculated with *Neorhizobium galegae* strain HAMBI 540 (University of Helsinki, Helsinki, Finland). The seeds of *N. galegae*-inoculated *G. orientalis* and *B. inermis* cv. 'Lehis' (Jõgeva Plant Breeding Institute, Estonia) were inoculated with two PGPB strains, *Pseudomonas trivialis* 3Re27 (Graz University of Technology, Graz, Austria) and *Pseudomonas extremorientalis* TSAU20 (National University of Uzbekistan) according to Egamberdieva et al. (2010), as the co-inoculation of these two PGPB strains with *N. galegae* were found to improve growth and symbiotic performance of fodder galega in a greenhouse experiment (Egamberdieva et al., 2010). PGPB-free seeds were used as controls. The seeds were manually sown and lightly covered by raking. Crops were harvested twice a year from 2010 on. Weeds were controlled manually. Soil samples were taken from the top 20 cm layer in the field at six time points (July 2009, May 2010, November 2010, May 2011, May 2012 and October 2012) and stored at −20 °C until the analysis. Soil chemical properties of three sample sets (July 2009, November 2010 and May 2012) were measured. Electrical conductivity (EC) and soil pH were measured in a 1:2.5 (v:v) soil–water suspension with MeterLab™ CDM210 (Radiometer Analytical) and SCHOTT CG842 pH-meter (SI Analytics), respectively. Soil dry matter content was determined by drying to constant mass at 105 °C. Soil total C and N contents were analyzed using the VarioMax CN-analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) and corrected to the dry-weight basis. The oil concentration in each oil-spiked plot was determined as the difference of total solvent extractable material (TSEM) concentration between the plot and the average of 4 to 5 randomly selected control plots at each sampling time. Detailed information on the field design, oil spike, soil sampling, measurements of soil chemical properties and TSEM determination are described in Yan et al. (2015).

2.2. DNA extraction and LH-PCR

Soil DNA was directly extracted from 0.50 g moist soil samples with FastDNA SPIN kit for Soil (Qbiogene, USA) according to the manufacturer's instructions. The final elution volume was 75–125 μL. The DNA yield of the first four sample sets was measured fluorometrically on a 96-well plate according to the manufacturer's instructions (PicoGreen dsDNA Quantification Reagent Kit; Molecular Probes).

Soil DNA extract was diluted 1/50 with sterile deionized water to avoid PCR inhibition by co-extracted humic substances in soil. Length heterogeneity PCR (LH-PCR) with 0.5–5 ng of DNA as a template was performed as described by Mikkonen et al. (2011b). The amplified fragments were separated with polyacrylamide capillary electrophoresis using ABI PRISM 310 Genetic Analyzer (Applied Biosystems).