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Oily waste containing natural radionuclides: does it cause stimulation or inhibition of soil bacterial community?

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Abstract

Contamination with oily wastes containing natural radionuclides is a potential hazard for soil health and function. Our study aimed to reveal both structural and functional changes of the microbial community resistant to and able to decompose oily wastes in soil. To do this, we determined CO_2 efflux, microbial biomass (by the extraction-fumigation method), and community structure (by PCR-SSCP) for 120 d after application of radioactive oily wastes to the soil at the ratio 1:4. The addition of the waste resulted in an increase of the activity concentration of ^{226}Ra by 130 times (up to 643 Bq kg^{-1}) and of ^{232}Th by 29 times (up to 254 Bq kg^{-1}). The calculated weighted dose for the radionuclide ^{226}Ra was found to be below the values that are known to affect microorganisms. However, the cumulative effect of a repeated deposition of radioactive oily waste may result in an increase of the weighted dose up to an effective level. During the incubation, the hydrocarbon (HC) content of the waste-treated soil decreased from 156 to 54 g kg^{-1} of soil indicating intensive decomposition of added organics by soil microorganisms. The waste application, however, led to an inhibition of soil microbial biomass compared with the control (by 26–47%). Microbial respiration was stimulated in the first month of incubation and then decreased until the end of the incubation period (by up to 74% compared to the control). The qCO_2 was estimated to be 3-fold higher than the control on day 1 of incubation and equal to the control on day 120 of incubation. The bacterial diversity decreased in the contaminated soil compared with the control soil. The bacterial community structure was altered by domination of new oil degrader species belonging to the genera *Dyella, Pseudoxanthomonas, Sinobacter*, and *Parvibaculum*. Thus, disposal of radioactive petroleum waste strongly altered the structure of the microbial community resulting in the selection of resistant species able to decompose pollutants and also affected the community function (inhibition of microbial biomass and stimulation of respiration) which tended to stabilize after long-term incubation.

Key words: oily waste / natural radionuclides / bacterial community structure / microbial biomass carbon / microbial respiration

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1 Introduction

Hydrocarbons (HC) contained in oil or oily waste being disposed in soil can be considered as a C source, especially for some specific microbial groups, but also as a contaminant which inhibits indigenous microflora. Considering that oily wastes can contain not only HC but also other toxic compounds (*Caravaca* and *Roldán*, 2003; *Gumerova* et al., 2014), the question of their effects on the soil microbial community is even more complex.

Oily waste is produced by two processes: the lubrication of equipment, such as pipelines, storage tanks, and pumps; or by the cleaning of petroleum residues which are precipitated on the walls of the pipes, reducing their flow rate and requiring periodic disposal (*El Afifi* and *Awwad*, 2005; *Abo-Elmagd* et al., 2010). As a result the large quantities of oily wastes (sludges or scales) may cause environmental pollution and simultaneously block storage spaces (*Godoy* and *da Cruz*, 2003; *Bakr*, 2010). The ecological consequences of such contamination need to be studied given that one ton of oily waste is produced for every 500 t of oil processed and about 60 Mt of oily waste are generated yearly (*Hu* et al., 2013). The petroleum wastes vary in concentrations of HCs (40–60%), water (30–90%), and mineral particles (5–40%) (*Lazar* et al., 1999). The total amount of HC in oily waste varies from 0.69 to 220 g kg^{-1} depending on the technology

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of oil processing (*Marin* et al., 2005; *Ros* et al., 2010). The petroleum hydrocarbons of oily waste contain alkanes, aromatics, resins, and asphaltene fractions (*Mishra* et al., 2001; *Tahhan* and *Abu-Ateih*, 2009). The mineral part of oily wastes often contains radioactive elements (such as radium, uranium, thorium, *etc*.). These so-called technically enhanced naturally occurring radioactive elements are dissolved in the water that is pumped up with the oil and then become concentrated in the oily waste (*El Afifi* and *Awwad*, 2005; *Selivanovskaya* et al., 2013; *Galitskaya* et al., 2014). The origin of these elements is the formation of both sulfate and carbonate precipitates inside petroleum production equipment, *e.g.*, Ba-sulfate complexes (*Doerner* and *Hoskins*, 1925; *Bakr*, 2010). The main radionuclides of oily wastes are isotopes of Ra (^{226}Ra and ^{228}Ra) and the typical activity concentration of radium is reported to range from 1 to 15,000 Bq kg^{-1} of waste (*EPA*, 1993; *Gazineu* et al., 2005; *El Afifi* et al., 2009).

Most investigations of such wastes are focused on the direct effect of radionuclides on human health (*El Afifi* and *Awwad*, 2005; *Bakr*, 2010). Less attention is paid to their impact on microbially-mediated decomposition of soil organic matter (OM). Radioactive elements of the oily waste may cause chromosomal aberrations, single strand breaks, and base pair substitution in the DNA of microorganisms (*Min* et al., 2003). Short chain HC may alter fluidity and permeability of microbial cell membranes, inhibit enzymes, disrupt the electron transport chain and oxidative phosphorylation, change cell homeostasis, *etc.* Long chain hydrocarbons may be toxic due to lipid proliferation (*Ruffing* and *Trahan*, 2014). Additionally, HC pollution can inhibit the microbial community indirectly by changing the aeration and watering conditions of soil microhabitats.

Despite the existence of various technologies applied for treatment of oily waste, they still often are directly disposed of on soil surfaces during road building in many countries including Russia (*Hejazi* et al., 2003). However, there is still a lack of knowledge of the effects of wastes containing both HC and natural radionuclides on soil and soil habitants.

Soil microorganisms play an essential role in the decomposition of OM and nutrition cycles. Microbial activity parameters are widely used to assess ecological health because they respond relatively quickly to contaminants and to the input of bioavailable organic compounds. Specifically, soil microbial biomass and basal respiration are commonly accepted community-level indicators of pollutant decomposition in the soil (*Selivanovskaya* and *Latypova*, 2006; *Tang* et al., 2011). The microbial biomass carbon amounts for only 0.5–4.6% of the soil TOC content, but soil microorganisms are the potential decomposers of compounds ensuring soil fertility (*Castorena-Cortés* et al., 2009; *Capelli* et al., 2001). The basal respiration reflects the activity of soil microorganisms and is considered as a useful sum indicator of the soil microbial activity (*Capelli* et al., 2001; *Dawson* et al., 2007). Microbial functioning altered by contamination is often related to changes in community structure (*Balba* et al., 1998; *Zhang* et al., 2010). The shifts in microbial community structure caused by pollutants can alter nutrient cycling in soil and thus cause changes in vegetation development, ecosystem functioning, and productivity (*Ros* et al., 2010). Although a decrease in the microbial diversity of petroleum-contaminated soils has already been reported, little is known about the effect of altered community structure on microbial functioning (*Li* et al., 2007; *Bastida* et al., 2010).

Our study aimed to evaluate the effects of oily wastes containing natural radionuclides on the structure, population size, and decomposition activity of soil microbial communities.

2 Material and methods

2.1 Field sites, waste and soil sampling, and preparation.

Waste sample (W) was collected from tanks at the Tikchonovskii petroleum production yard (Tatarstan, Russia) (54°50′26′′ N, 52°27′08′′ E) during cleaning and maintenance routines. The soil (Luvisol) was sampled from the upper 0–20 cm at the forest-nursery "Matyushenski" in Tatarstan, Russia (55°48′07′′ N, 49°16′13′′ E). Immediately after sampling, the soil was mixed thoroughly and passed through a 5-mm sieve to remove large roots and plant residues. The soil and waste properties are presented in Table 1.

Table 1: Chemical and physical properties of the soil and waste used in the experiment.

Parameter	Soil	Waste
C_{org} / g kg^{-1}	18.2 ± 2.3	nd
N_{total} / mg kg^{-1}	1100 ± 286	nd
$K_{extractable}$ / g kg^{-1}	91 ± 13	nd
$P_{extractable}$ / g kg^{-1}	125 ± 17	nd
pH (H_2O)	7.2 ± 0.4	nd
TPH / g kg^{-1}	0.4 ± 0.03	720.5 ± 21.46
aromatics / %	nd	36 ± 2
aliphatics / %	nd	16 ± 3
resins / %	nd	21 ± 3
asphaltenes / %	nd	27 ± 5
activity concentrations / Bq kg^{-1}		
^{226}Ra	21 ± 2	2739 ± 180
^{232}Th	32 ± 2	916 ± 56
^{40}K	311 ± 17	271 ± 51
particle size distribution / %		
sand	31.3	nd
silt	66.0	nd
clay	2.7	nd

[a]nd: not determined.

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2.2 Experiment design and samples analysis

The soil was thoroughly mixed with the waste at a ratio of 4:1 to obtain a homogenized mixture (WS). Untreated soil was used as the control (C). In order to estimate the functioning of microbial community of the initial waste it was also used in experiment (W). The laboratory incubation was performed in triplicate at 25°C and 60% of water-holding capacity for a period of 120 d. The treated (WS) and control (C) soils, and the initial waste (W) were placed in glass pots (2 kg of WS, C, and W per pot), and distilled water was added to reach 60% of the soil water-holding capacity. The samples in the pots were mixed thoroughly every 3 d, and 10 sub-samples from the treated and control soils and from the waste were collected on days 1, 30, 90, and 120. The sub-samples were thoroughly mixed to obtain a representative and homogenized 60 g samples. The samples were placed in sterile plastic bags, sealed, and stored at 4°C for chemical, physical, and microbial analyses or at –20°C for molecular analysis.

Soil pH was measured in an aqueous soil extract in deionized water (1:2.5 soil:water) according to *ISO 10390:2005* (2005).The total organic C content (TOC) was determined by sulfochromic oxidation according to *ISO 14235:1998* (1998). Total N (Ntot) was determined using the modified Kjeldahl method according to *ISO 11261:1995* (1995). Water holding capacity (WHC) was determined according to *ISO 11274:1998* (1998). Available K was determined by an extraction method with NH_4-acetate (*Pratt*, 1965), and available P by extraction method with $NaHCO_3$ solution (*Olsen*, 1954). Particle size analysis was carried out using the laser diffraction method according to *ISO 13320:2009* (2009). The HC content of the waste and soil samples was determined by IR spectrometry using an AN-2 analyzer (LLC "NEFTEHIMAVTOMATIKA-SPb", Saint-Petersburg, Russia). The natural radioactivity (^{226}Ra, ^{232}Th, and ^{40}K) of waste samples was analyzed by γ-spectroscopy. The samples were dried in an oven at 110°C for 24 h, homogenized, and sieved through 0.8-mm mesh. The sieved samples were weighed and packed in a Marinelli-type beaker (1000-ml capacity) until their analysis using a gamma spectrometer "Progress" (SPC "Doza", Zelenograd Moscow, Russia). The samples were carefully sealed and stored for 4 weeks to achieve secular equilibrium between ^{226}Ra and its progenies. The gamma-ray spectrometric measurements were performed using a scintillation block which was based on a crystal of Na-Tl-iodide and a "Progress" gamma-spectrometer.

The soil microbial biomass (C_{mic}) was determined after the fumigation of the samples using ethanol-free $CHCl_3$ and extraction with 0.5 M K_2SO_4. The extracted C content was determined through dichromate oxidation in accordance with *ISO 14240-2* (1997). The basal respiration rates (CO_2 production from unamended soil) were determined in 10 g soil samples during 24 h. The CO_2 trapped in 1 M NaOH solution was precipitated with 0.5 M $BaCl_2$ and then the excess of NaOH was titrated with 0.2 M HCl using the phenolphthalein indicator (*Schinner* et al., 1995). The metabolic quotient (qCO_2) was calculated by dividing the C-CO_2 evolution rate by the microbial biomass C contents.

DNA was extracted using a FastDNA® SPIN kit for soil (Bio101, Qbiogene, Germany) according to the provided instructions, and the concentration of DNA was measured using a spectrometer at 260 nm (Thermo Scientific GENESYS 20™, Thermo Fisher Scientific Inc., Waltham, USA). The DNA extracts were stored at –20°C overnight until further analysis. The fingerprinting of the bacterial communities by single strand conformation polymorphism (SSCP) was performed in two replicates as described by *Kampmann* et al. (2012). Briefly, polymerase chain reaction (PCR) was performed using a thermocycler (MyCycler, Bio-Rad, Munich, Germany). The chemicals and enzymes for PCR were purchased from Fermentas (St. Leon-Rot, Germany). The reaction mixture (total volume of 50 μL) contained 0.02 U-define μL^{-1} Dream*Taq* DNA Polymerase, 1x Taq Buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTPs, 0.2 μmol^{-1} of each primer, 0.16 mg mL^{-1} BSA, and 2 μL of extracted DNA. The bacterial communities were analyzed using the universal bacterial 16S rRNA primer pair Com1/Com2 (CAG CAG CCG CGG TAA TAC / CCG TCA ATT CCT TTG AGT TT) (*Schwieger* and *Tebbe*, 1998). The primers were purchased from Eurofins MWG Operon (Ebersberg, Germany). The PCR was performed as follows: 95°C for 3 min, 16 cycles of 94°C for 30 s, 64 to 57°C for 30 s, and 72°C for 30 s, 9 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 30 s, and a final elongation step at 72°C for 30 min. The PCR products were purified using a QiaQuick PCR purification kit (Qiagen). Before electrophoresis, ssDNA fragments were generated by lambda exonuclease digestion (*Schwieger* and *Tebbe*, 1998). The ssDNA was separated using an INGENYphorU electrophoresis system (Ingeny International BV, Netherlands) at 450 V and 19.5°C in a non-denaturating polyacrylamide gel with a 0.6x MDE solution (Biozym Scientific GmbH, Hessisch Oldendorf, Germany) and 1x TBE buffer (0.89 M Tris, 0.89 M boric acid, and 20 mM EDTA pH 8.0) for 17 h. The gel was then silver-stained using a Page silver staining kit (Fermentas, Germany). The silver-stained SSCP gel was scanned to obtain digitized gel images. The dominant bands were excised from the SSCP gel as described by *Schwieger* and *Tebbe* (1998). The gel-extracted DNA was reamplified and cloned as described by *Kampmann* et al. (2012) using the pGEM-T® Vector System (Promega, Mannheim, Germany). Four clones of each band were sequenced. All of the sequencing was performed by LGC Genomics. For sequencing, the forward primer M13 (Promega) was used and the clones to be sequenced were sent to LGC Genomics GmbH (Berlin, Germany) in a 96-well microtiter plate which was filled with LB (Lysogeny Broth)-agar and 50 $\mu g\ mL^{-1}$ ampicillin. The quality checks and cutting of the sequences were performed using the software package MEGA, version 5.0 (*Tamura* et al., 2011). The sequences were analyzed for chimeras using the Pintail program (Version 1) (*Ashelford* et al., 2005), and putative chimeras were removed from the dataset. The alignments were performed with the SILVA web aligner (*Pruesse* et al., 2007), and the similarity values were calculated using the neighbor joining algorithm PHYLIP (*Felsenstein*, 1989), which was implemented using the ARB software package (*Ludwig* et al., 2004). For the sequence comparisons, the SILVA SSU 106 Ref database was used.

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The sampling and the chemical and biological analyses were conducted in triplicate. The results are expressed on an air-dry soil basis. The data from the experiments were processed using statistics package and of Origin 8.5 (OriginLab, Northampton, USA). The means were compared using Fisher's Protected Least Significant Difference at $\alpha = 0.05$. The values in the figures and tables were expressed as the means ± S.E. of the corresponding replicates.

3 Results

The HC content in waste was estimated to be 721 ± 21 g kg^{-1} (Fig. 1a). It did not change significantly with time. After mixing of the waste with the soil, the level of TPH in the soil increased up to 156 ± 48 g kg^{-1}, so the soil was classified as heavily polluted (*Al-Awadhi* et al., 1996).

HC degradation in the spiked soil was faster during the first month when 20% of the added HC was decomposed. Thereafter, degradation was slower reaching 65% after 120 d of incubation. The concentration of radionuclides in the contaminated soil was estimated to be 643 ± 127 and 254 ± 56 Bq kg^{-1} for ^{226}Ra and ^{232}Th, respectively, and was not significantly changed after 120 d of incubation. Using the spreadsheet-based model R&D128 (Terrestrial model) from the England and Wales Environment Agency (*Coppelstone* et al., 2001; *Jones* et al., 2003) the weighted dose for the radionuclide ^{226}Ra was calculated for the bacteria in the soil sample studied. For ^{226}Ra, the weighted dose rate was 152 μGy h^{-1}. The total dose over the experiment duration was 0.4 Gy for the ^{226}Ra radionuclide.

The microbial biomass in control soil on the first day of the experiment was found to be 672 mg C_{mic} kg^{-1} and did not change significantly during the experiment (Fig. 1b). In the waste, the level of microbial biomass was much lower –3.1 ± 0.2 mg C_{mic} kg^{-1} and remained low during the incubation period. In the waste-treated soil, almost immediately after spiking microbial biomass decreased by the factor of two. By the end of the incubation period microbial biomass increased, thus, the difference between its level in the waste-treated soil and the control soil decreased.

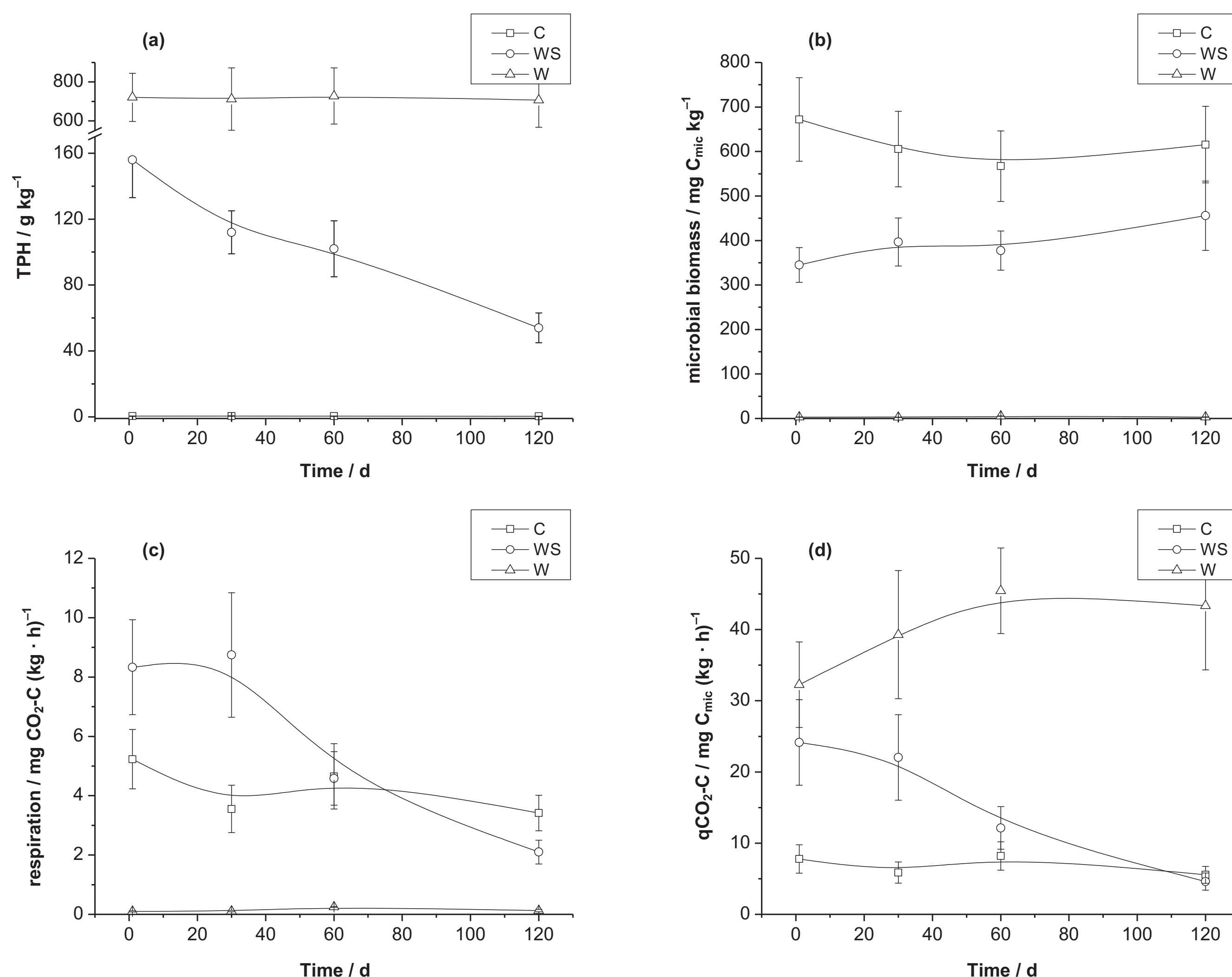

Figure 1: Evolution of parameters of waste (W), soil treated with the waste (WS), and control soil (C) during the incubation experiment. (a) Total hydrocarbon (HC) content; (b) microbial biomass; (c) respiration; (d) metabolic quotient.

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Microbial respiration in the control soil ranged between 3.4 and 5.2 mg CO_2-C $(kg \cdot h)^{-1}$, while in the W samples it ranged between 0.1 and 0.3 mg CO_2-C $(kg \cdot h)^{-1}$ (Fig. 1c). The amounts of CO_2 that evolved from the WS samples after 1 and 30 d of incubation were approx. 160 and 250%, respectively, as compared to control soil. In the course of the incubation, microbial respiration decreased to values similar (at day 60) or even below those in the control soil at day 120.

The metabolic quotient of the control soil varied from 5.5 to 8.2 mg CO_2-C $(mg\ C_{mic} \cdot h)^{-1}$ during the incubation period (Fig. 1d). The values of qCO_2 increased significantly in soil after the addition of waste: they were 3.1- and 3.8-fold higher than those of the control soil after 1 and 30 d of incubation, respectively. This increase can be attributed to the increase in microbial respiration (Fig. 1c) and simultaneous decrease in microbial biomass (Fig. 1b) observed during this period in the treated soil. During the subsequent months, the values of qCO_2 of the treated soils decreased and were similar to the control values at the end of the incubation period. The respiration rate and metabolic quotient were in accordance with the decrease in the HC content during the experiment (R = 0.86 and 0.92, respectively).

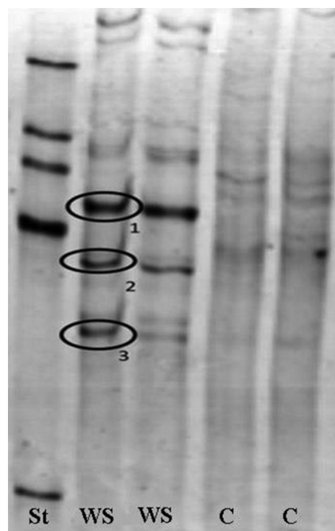

Figure 2: SSCP analysis of control (S) soil, and of soil treated with waste (SW) at the day 120 of incubation. St: bacterial standard, as described by *Kampmann* et al. (2012). Circled bands were cut out, and DNA was isolated for cloning and subsequent sequencing.

In SSCP profiles of unpolluted soil no dominant bands were observed (Fig. 2). However, three dominating strains were revealed in the SSCP profile of the waste polluted samples.

These bands (1, 2, and 3) were excised and sequenced. All of the cloned sequences from band 1 (Pol 7-a, f, j, and k) were closely (98.8 to 99%) related to the genus *Dyella* (EU872214; EF191354) (Fig. 3). Three clones from band 2 were closely related to *Sinobacter flavus* (EF154515; 99.3 to 100%; Pol 5-c, f, and j), and one clone was closely related to *Pseudoxanthomonas spadix* (AM418384; 99.5%). The clone sequences from band 3 (Pol 8-g and Pol 8-j) were distantly related to *Parvibaculum lavamentivorans* (AY387398; 95.7%) and sediment bacterium 22-39 (EU167984; 99.8%).

4 Discussion

4.1 Changes in the chemical composition of the soil polluted by the oily waste

The activity concentrations of ^{226}Ra, ^{232}Th, and ^{40}K in the soil used in our experiment were comparable with those of the natural background of the soil (*Starkov* and *Migunov*, 2003) (Table 1). The radioactivity in the analyzed waste was within the range found previously in sludges from oil production and processing plants (*Bakr*, 2010), much lower than the levels of ^{226}Ra and ^{40}K found in scales and sludges generated during oil extraction and production operations (*Shawky* et al., 2001; *Gazineu* and *Hazin*, 2008; *Abo-Elmagd* et al., 2010), and higher than their levels in scales and sludges from refinery exchangers and old gasoline tanks (*Shawky* et al., 2001; *Al-Saleh* and *Al-Harshan*, 2008). These differences can be explained by the initial

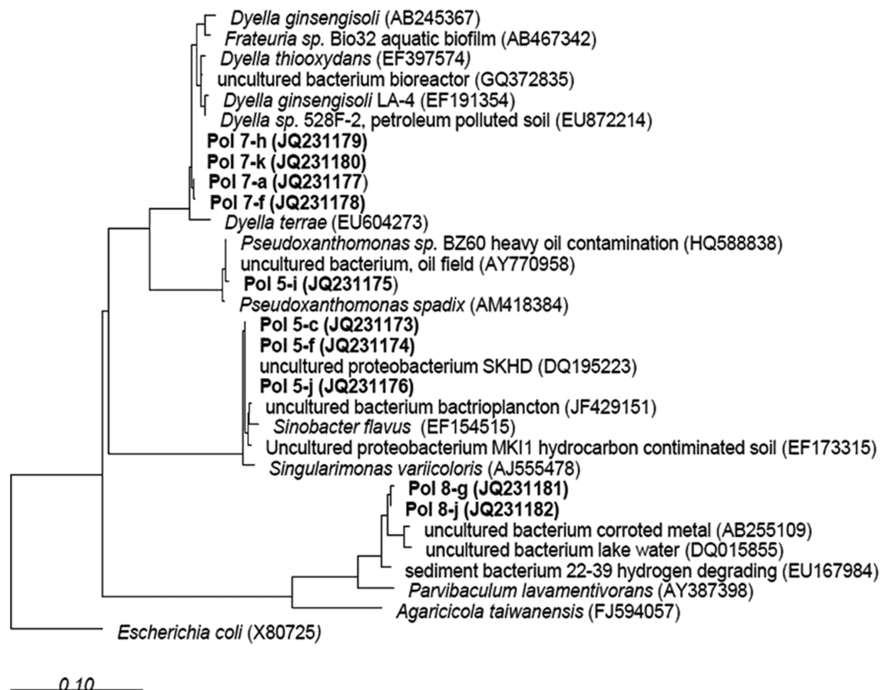

Figure 3: Phylogenetic tree of 16S rRNA partial gene sequences from soil treated with oily waste (DNA was sampled on the day 120 of incubation). The tree was constructed using the maximum likelihood algorithm for the nearly full-length sequences (> 1,400 bp). Clone sequences (in boldface type) from the SSCP-DNA bands were added using the ARB parsimony tool. GenBank accession numbers are also in parentheses. *Escherichia coli* (X80725) was used as the outgroup.

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content of natural radionuclides in the rocks from which the oil was extracted and by differences in the oil processing technologies. The activity concentrations of the studied isotopes were below the exempt level of International Basic Safety Standards for naturally occurring radioactive materials (10,000 Bq kg^{-1} for ^{226}Ra and 1000 Bq kg^{-1} for ^{232}Th) (*IAEA 11511*, 2001). However, the radioactivity levels of the waste were much higher than those found for control soil, indicating that this waste can significantly increase the radioactivity of the soil. Indeed, after the addition of the waste to soil, the levels of activity concentration of ^{226}Ra and ^{232}Th increased 36- and 9-fold, respectively. The range of doses that have been found to affect microorganisms is above 40 Gy (*Jones* et al., 2004) or even 50 kGy (*Yardin* et al., 2000; *Buchan* et al., 2012). Thus, in our experiment the calculated weighted dose for the radionuclide ^{226}Ra was far below the values that are known to affect microorganisms, which are the least irradiation-sensitive organisms. However, the cumulative effect of a repeated deposition of radioactive oily waste might result in an increase of the weighted dose up to an effective level.

The HC content in the waste was comparable with the levels found in other studies (*Marin* et al., 2005; *Liu* et al., 2009; *Roldán-Carrillo* et al., 2012; *Hu* et al., 2013).

Relatively slow HC degradation in the waste-treated soil (about 30% in the first two months and about 65% in 4 months) is explained by the 3–4 times higher oily sludge contamination as compared with fast biodegradation of TPH in soil with low-level contamination (*Marin* et al., 2005). Another explanation may be the chemical composition of the waste. In our case, the dominant components of the waste were aromatics and asphaltenes (Table 1), which are more recalcitrant and less accessible to microorganisms in comparison with aliphatics (*Marin* et al., 2005).

4.2 Changes in the microbial properties of the soil polluted by the oily waste

Oil HC, depending on their concentration and chemical composition, can lead to a stimulation of microbial biomass growth by serving as a carbon source or to its inhibition because of their toxic properties (*Marin* et al., 2005; *Dawson* et al., 2007). Both increasing and decreasing trends of the toxicity of oil HC in soil over time have been reported (*Caravaca* and *Roldán*, 2003; *Plaza* et al., 2005). The decrease in soil microbial biomass immediately after the addition of waste could be a result of inhibitory properties of the waste towards microorganisms as well as a result of simple dilution of the soil by the waste. Taking into account the low level of biomass in the waste (3.1 mg C_{mic} kg^{-1}), we calculated the level of biomass of the soil after its dilution with the waste—it would be approx. 550 mg C_{mic} kg^{-1}. In our case the estimated level is 1.6-fold lower than the calculated one. Thus, the waste can be considered mainly as a contaminant for soil microorganisms in the beginning of the incubation. Over time, the difference between microbial biomass in the waste-treated soil and the control soil decreased. This coincides with the decrease of HC content. The increase of microbial biomass by the end of experiment may be explained by degradation of oil hydrocarbons or by decrease of toxicity of HC and their metabolites (*Labud* et al., 2007; *Al-Mutairi* et al., 2008). Another explanation for the increase in microbial biomass is the replacement of soil indigenous species by HC oxidizing microbes (*Li* et al., 2007). This explanation is supported by a significant increase in counts of HC oxidizing bacteria in the first two months of incubation (data not shown).

The incorporation of waste into the soil greatly increased microbial respiration in the first 30 d. There are several possible explanations for this observation: microbes were able to immediately degrade these compounds as new sources of C (*Marin* et al., 2005; *Labud* et al., 2007). This increase in respiration can be caused not only by the mineralization of HC or dead cell biomass but also as a result of a stress response of the soil microorganisms to contamination (*Chander* and *Brookes*, 1991; *Franco* et al., 2004).

The metabolic quotient which reflects carbon use efficiency as well as carbon availability and microbial maintenance requirements (*Blagodatskaya* and *Kuzyakov*, 2013) was used in this study as an ecophysiological index (*Anderson* and *Domsch*, 2010). In polluted sites the qCO_2 levels can be used as an index of stress due to contamination (*Anderson* and *Domsch*, 1990; *Caravaca* and *Roldán*, 2003). According to the qCO_2 values, the maximal disturbance of the microbial community was observed immediately after contamination. Over time, the community adapted to the stress through functional and structural changes with a corresponding decrease in qCO_2. At the end of the experiment, the qCO_2 value was similar to that of the control indicating stabilization of the microbial community after decomposition of the pollutant. A remarkably high qCO_2 level was observed during the 120 d of incubation in the sole waste sample in which almost no decomposition of HC was detected.

The changes in microbial community functioning in the waste-treated soil can be related to the shift of microbial community structure, which we revealed by the SSCP fingerprinting. Since bacteria are reported to be more effective oil oxidizers compared with fungi (*Máthé* et al., 2012), we have focused in this study on shifts in bacterial community under influence of oily waste. In 120 d of incubation bacterial genetic diversity decreased in polluted *versus* original soil. A similar effect for soil caused by oily products was demonstrated by *Li* et al. (2007). Additionally, several dominating strains were observed in the bacterial community of the spiked soil compared with the control soil. These dominant strains were phylogenetically close to strains of the genus *Dyella, Pseudoxanthomonas, Sinobacter*, and *Parvibaculum*, which have been previously described to be HC-oxidizing or oil-tolerant. Thus, the strain *Dyella* sp. 528F-2 related to excised strain 1 has been found in petroleum-polluted soil in an oil field (Dongying, Shandong, China), and *Dyella ginsengisoli* strain LA-4 has been isolated as a biphenyl degrader. Biphenyl is a component of crude oil (*Li* et al., 2009). *Pseudoxanthomonas spadix* (*Young* et al., 2007), *Pseudoxanthomonas* sp. BZ60, and the DNA sequence from an uncultured bacterium related to one clone from band 2 have been isolated from oil-contaminated soils or oil fields (*Chang* et al., 2005; *Patel* et al., 2012). Other clone sequences of band 2, that were related to *Sinobacter flavus*, showed high similarity to the sequence of uncultured

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Proteobacteria MKl1 which was retrieved from HC-contaminated soil (*Low* et al., 2007). The clones from band 3 were closely related to *Parvibacullum lavamentivorans* which is able to metabolize omega-oxygenate, the commercial surfactant linear alkylbenzene sulfonate (*Schleheck* et al., 2004) and to the sediment bacterium 22-39 that was isolated as a polycyclic aromatic HC-degrading bacteria from the Elizabeth River sediments (*Hilyard* et al., 2008). Changes in bacterial diversity can be explained by selective pressure of the soil conditions caused by an increase in compounds toxic towards bacteria and the presence of non-typical C substrates.

Thus, 120 d incubation of soil treated with oily waste containing natural radioactive elements led to the alteration of the microbial community. This alteration was expressed as a simultaneous inhibition of microbial biomass, a stimulation of respiration and increase in qCO2, and also in a shift in the structure of microbial community towards dominant strains which are resistant to HC pollution.

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