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Suppression of soil organic matter decomposition by gasoline and diesel as assessed by ^{13}C natural abundance

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ABSTRACT

Petroleum products are common contaminants in soils due to human activities. They are toxic for microorganisms and threat their functions, including decomposition of soil organic matter (SOM). The direct estimation of altered SOM decomposition – based on the CO_2 efflux – is impossible after petroleum contamination because petroleum decomposition also contributes to these CO_2 fluxes. We used the natural differences in the isotopic signature ($\delta^{13}C$) of SOM and of petroleum products to partition the total CO_2 for both sources and to analyse the suppression of SOM decomposition. The dynamics of ^{13}C fractionation during the mineralization of gasoline and diesel was measured during 42 days. The ^{13}C fractionation varied between −8.8‰ and +3.6‰ within the first 10 days, and stabilized thereafter at about −5.3‰ for gasoline and +3.2‰ for diesel. These ^{13}C fractionations and $\delta^{13}C$ values of CO_2 emitted from the soil were used to partition the total CO_2. Contamination with gasoline reduced the CO_2 efflux from SOM by a factor of 25 (from 151 to 6.1 mg C-CO_2 kg^{-1} soil during 42 days). The negative effect of diesel was much lower: the CO_2 efflux from SOM was decreased by less than a factor of 2. The strong effect of gasoline versus diesel reflects the lower absorption of gasoline to mineral particles and the development of a thin film on water surfaces, leading to toxicity for microorganisms. We conclude that the soil contamination by gasoline and diesel strongly decreased microbial functions and so, the degradation of native SOM.

1. Introduction

Petroleum compounds are frequent and significant pollutants of soils in many areas, especially at oil production and processing sites [1–3]. One of the few and comparatively inexpensive ways to reduce the concentration of such organic pollutants is their decomposition by native soil microorganisms. This decontamination approach is useful from both the environmental and economic standpoints [4–6]. The intrinsic microbial biodegradation of petroleum products involves their mineralization, transformation into nontoxic compounds, or long-term bonding on mineral particles and soil organic matter. Certain microorganisms can use petroleum compounds as a main source of carbon and electrons [7]. Numerous *in situ* studies have enabled selecting the most active microorganisms capable of biodegrading aliphatic and aromatic petroleum compounds. These include inter alia: *Planomicrobium chinense*, *Rhodococcus erythropolis*, *Micrococcus luteus*, *Pseudomonas putida*, *Pseudomonas fluorescens* and *Mycobacterium frederikbergense* [8–10].

Various parameters were measured in studies on biodegradation of petroleum compounds in soils, including degradation rates, microbial groups involved, changes in toxicity and completeness of decomposition of toxic substances. The most frequently used approaches are: i) analysis of enzyme activities, e.g. dehydrogenase activity [9]; ii) microbiological analysis of cell morphology, conidiophores and conidies, motility, Gram-reaction [11]; iii) chromatographic analysis of petroleum components and

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transformation products, e.g. using gas chromatograph coupled to a Polaris Q® mass spectrometer [12].

One recently suggested and rapidly developing approaches to investigate the decomposition of petroleum compounds in soils is to analyse the isotopic composition ($\delta^{13}C$) of released CO_2 [13–15]. This approach is intensively used especially in aquatic and soil environments to track and assess contamination by petroleum products [16,17]. It is based on natural differences in the isotopic composition ($\delta^{13}C$) of carbon of petroleum and of soil organic matter. Thus, based on the CO_2 release and its $\delta^{13}C$ values from petroleum and SOM, it is possible to distinguish between the CO_2 originating from both sources. Nonetheless, most previous studies have not considered ^{13}C fractionation by decomposition of petroleum products and mineralization to CO_2. Therefore, the results of these studies may be biased.

The present study was designed to determine the effect of petroleum products on soil microbial activity for the decomposition of SOM. For this purpose, CO_2 released from soil contaminated by petroleum products was partitioned for C sources using the $\delta^{13}C$ values of CO_2. One focus was on the dynamics of ^{13}C fractionation during decomposition because the ^{13}C fractionation may change depending on the products that are being mineralized in a specific period [18,19]. We used these results to assess how rapidly native soil microorganisms begin to degrade these organic contaminants.

2. Material and methods

2.1. Experimental layout

The experiment consisted of five major treatments. The 1st treatment – Control, incubation of soil without any addition – was necessary to analyse the CO_2 and its $\delta^{13}C$ produced by decomposition of native soil organic matter. The 2nd and 3rd treatments were introduced to analyse ^{13}C fractionation by decomposition of petroleum products. For this, gasoline and diesel were incubated in sand after adding microbial inoculum from soil. The 4th and 5th treatments involved incubation of soil with the same petroleum products. Sand treatment (without petroleum products) was not incubated because preliminary experiments showed that the CO_2 efflux from it is below the detection limit.

2.2. Soil and sand

Soil was sampled from the arable horizon (0–30 cm) of a Haplic Umbrisol in Złota (Świętokrzyskie Voivodeship, central Poland) [20]. The basic properties of the soil are shown in Table 1.

Partitioning CO_2 sources by $\delta^{13}C$ requires having the values of ^{13}C fractionation by petroleum mineralization. We therefore used incubation in sand with a very low content of other organic carbon. The sand was sampled from Świbno (southern part of the Gulf of Gdańsk, Baltic Sea).

Particle size distribution was measured with a Mastersizer 2000 with HydroG dispersion unit using the laser diffraction method [21,22]. Total organic carbon (TOC) was measured using TOC-V_{CPH} (Shimadzu) with SSM 5000A. For this, 300 mg soil covered by ceramic fibre was burned at 900 °C and the amounts of CO_2 produced were defined by infrared detection (NDIR). There was no inorganic C in the soil.

2.3. Petroleum products

Two of the most common petroleum products were used in the experiment: unleaded 95-octane gasoline produced by Orlen (Polish oil company) and diesel from BP. The $\delta^{13}C$ values were: for gasoline $\delta^{13}C = -31.12 \pm 0.10‰$, and for diesel $\delta^{13}C = -31.06 \pm 0.11‰$. The $\delta^{13}C$ values were measured in 9 replications.

2.4. Contamination procedure

The air-dried soil and sand were sieved through a 2 mm. 10 g of either soil or sand were weighed into dark glass bottles (60 cm^3) and moistened to pF 2.2 (i.e. field water capacity). The bottles were closed with butyl septa and aluminium caps. Then the contaminants were added by syringe through the septa. The experiment consisted of 5 treatments: 1) control – uncontaminated soil with addition of 0.2 ml distilled water; 2) sand contaminated by 0.2 ml gasoline; 3) sand contaminated by 0.2 ml diesel; 4) soil contaminated by 0.2 ml gasoline; 5) soil contaminated by 0.2 ml diesel. The high amount of petroleum products simulated the ecological disaster when gasoline or diesel are released from tanks.

All soil and sand samples were incubated in the dark in air-tight bottles (60 cm^3) at 25 °C. The experiment was done with 3 replications.

2.5. CO_2 and $\delta^{13}C$ analyses

The CO_2 concentrations and isotopic ratios were measured during the incubation on days 1, 3, 5, 7, 10, 14, 21, 28, 35 and 42 after addition of petroleum products. The CO_2 was accumulated between the samplings in closed bottles.

The CO_2 concentrations were measured using a Shimadzu GC-14A gas chromatograph with Thermal Conductivity Detector (TCD). Helium was used as the carrier gas. The GC was equipped with a steel column with Porapak Q. 50 μl of the gas was injected into the GC.

The $\delta^{13}C$ of CO_2 was measured using an Isotope Ratio Mass Spectrometer (IRMS) DELTA V Advantage (Thermo Scientific company) with Continuous Flow mode. The air volume (air from incubation containers) depended on the CO_2 concentration and ranged from 1 μl to 100 μl of air.

Samples for the GC and IRMS analysis were injected using a

Table 1
Selected soil and sand properties.

Parameters	Soil – Haplic Umbrisol	Sand
Site of sampling	Złota	Świbno
Texture	silt loam	sand
Particle size distribution	19.55%; (sand fraction: 2-0.05 mm) 73.20%; (silt fraction: 0.05-0.002 mm) 7.25%; (clay fraction <0.002)	42.16%; (coarse sand 1-0.5 mm) 57.66%; (medium sand 0.5-0.25 mm) 0.18%; (fine sand 0.25-0.1 mm)
TOC (%)	1.06	0.13
N content	0.16	0.01
C/N	6.63	13
$\delta^{13}C$ (‰)	−22.43	−20.56