European Journal of Soil Biology 73 (2016) 8–14

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

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

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ARTICLE INFO

Article history:
Received 11 August 2015
Received in revised form
14 December 2015
Accepted 18 December 2015
Available online xxx

Keywords:
Biodegradation
Soil remediation
CO_2 efflux
Petroleum hydrocarbons
^{13}C fractionation
$\delta^{13}C$ values

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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http://dx.doi.org/10.1016/j.ejsobi.2015.12.009
1164-5563/

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)	42.16%; (coarse sand 1-0.5 mm)
	73.20%; (silt fraction: 0.05-0.002 mm)	57.66%; (medium sand 0.5-0.25 mm)
	7.25%; (clay fraction <0.002)	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

syringe with a capacity of 100 μl (VICI company), with puschbutton valve and needle with conical tip and a side outlet. This ensured isolating the gas taken from the sample from the air in the laboratory.

The isotope ratios of petroleum products were determined in the 10 μl samples after their burning in glass tubes (20 cm long and 0.9 cm diameter) at 560 °C in the presence of copper II oxide (CuO). The released CO_2 was purified from water vapour and other impurities, and $\delta^{13}C$ values were measured using a triple-collector mass spectrometer with a dual-inlet system. The measurement was carried out in 9 replications.

2.6. Calculations and statistical analyses

After adding the petroleum products to soil, the isotope mass balance for CO_2 evolved by microbial mineralization of SOM and the petroleum products was calculated using the following equation:

$$\delta^{13}C^T \cdot C^T = \delta^{13}C^{soil} \cdot C^{soil} + \delta^{13}C^{pet} \cdot C^{pet}, \quad (1)$$

where:

$$C^T = C^{soil} + C^{pet}. \quad (2)$$

C^T stands for the amounts of CO_2 derived from the contaminated soil (soil with petroleum products). C^{soil} and C^{pet} stands for the amounts of CO_2 derived from the not contaminated soil (without petroleum products) and from the petroleum products, respectively. $\delta^{13}C^{soil}$ and $\delta^{13}C^{pet}$ are the $\delta^{13}C$ values of CO_2 emitted from the not contaminated soil and from petroleum products, respectively. $\delta^{13}C^T$ is the $\delta^{13}C$ value of CO_2 emitted from the soil contaminated with petroleum products.

Dividing Eq. (1) by C^T we obtain:

$$\delta^{13}C^T = \delta^{13}C^{soil} \cdot \frac{C^{soil}}{C^T} + \delta^{13}C^{oil} \cdot \frac{C^{pet}}{C^T}, \quad (3)$$

where C^{soil}/C^T and C^{pet}/C^T we denote by f^{soil} and f^{pet}, respectively. These are the portions of CO_2 from the soil and from the petroleum products, thus:

$$f^{soil} + f^{pet} = 1. \quad (4)$$

Using Eq. (4), we can write:

$$\delta^{13}C^T = \delta^{13}C^{soil} \cdot f^{soil} + \delta^{13}C^{pet} \cdot \left(1 - f^{soil}\right), \quad (5)$$

yielding:

$$f^{soil} = \frac{\delta^{13}C^T - \delta^{13}C^{pet}}{\delta^{13}C^{soil} - \delta^{13}C^{pet}} \quad (6)$$

The fraction of CO_2 resulting from petroleum product oxidation during the time interval can be calculated as:

$$I(CO_2)_i = \left(1 - f_i^{soil}\right) \cdot \Delta CO_{2_i}^{*} \quad (7)$$

where: ΔCO_2^{*} – rate of CO_2 evolved during the time interval [in mg C kg^{-1} dried soil day^{-1}], i – days of incubation (i = 0, 1, 3, 5, …).

The cumulative CO_2 produced during the microbial substrate oxidation was calculated using the equation:

$$Q = \sum I(CO_2)_i \quad (8)$$

The significance of differences between control and sample was analysed by Students t-test and was accepted at 0.05 level. The presented values reflect means of 3 replications ± standard error (SE).

3. Results

3.1. CO_2 fluxes

The microbial respiratory response strongly depends on the contaminant type. In the first step, we tested the reaction of soil microorganisms to the addition of petroleum compounds. The smallest CO_2 fluxes were measured in the control – only distilled water added to soil – where about 151 mg C-CO_2 kg^{-1} soil were released during 42 days of incubation (Fig. 1). Adding diesel to the soil caused the largest increase of CO_2 production. About 407 mg C-CO_2 kg^{-1} soil were released during 42 days of incubation. For comparison, adding gasoline caused CO_2 emissions of about 267 mg C–CO_2 kg^{-1} soil.

Contamination by diesel increased the CO_2 efflux until day 35 (Fig. 1). CO_2 efflux decreased significantly after 35 days (Fig. 2). Nonetheless, compared with gasoline contamination, where stabilization occurred at a much lower level, increased CO_2 efflux rates were measured only up to the 7th day. The dynamics of the CO_2 efflux from soil contaminated with gasoline show a peak on the 3rd day, when the value was 70 mg C–CO_2 kg^{-1} day^{-1}.

Addition of new C sources (gasoline and diesel) increased the CO_2 efflux from the soil. During the whole incubation period the total amount of the CO_2 almost doubled and almost tripled compared to the control for gasoline and for diesel, respectively. The amounts of total CO_2 show the overall decomposition of all organics in soil, but did not enable evaluating the contribution of individual C sources.

3.2. Isotopic signature of CO_2 and decomposition rates of petroleum products

The contribution of both CO_2 sources – soil organic matter and petroleum products – were analysed based on the $\delta^{13}C$ of released CO_2. Adding distilled water to the control soil increased the $\delta^{13}C$ value of CO_2 in the first 3 days of incubation (from −36.7‰), and thereafter it stabilized at a level of about −29.4‰ (Fig. 3).

The $\delta^{13}C$ values of CO_2 released from soils contaminated with petroleum products differed (Fig. 3). Gasoline caused a rapid

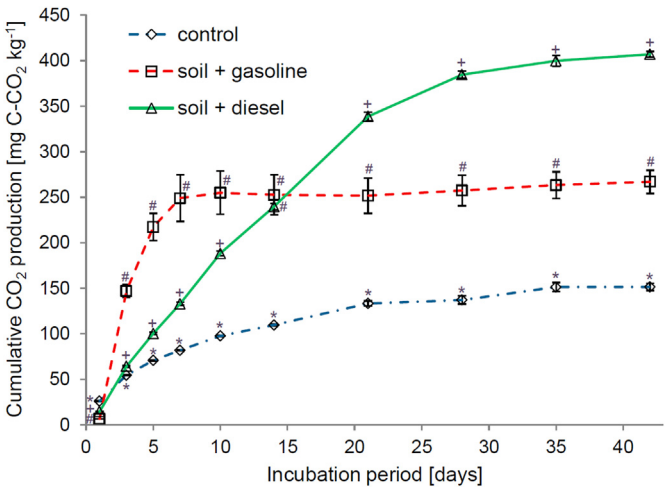

Fig. 1. Cumulative CO_2 evolved from soil contaminated with petroleum products. Whiskers show standard errors of the means (n = 3). For each time point, treatments with the same symbol are not significantly different ($p < 0.05$).

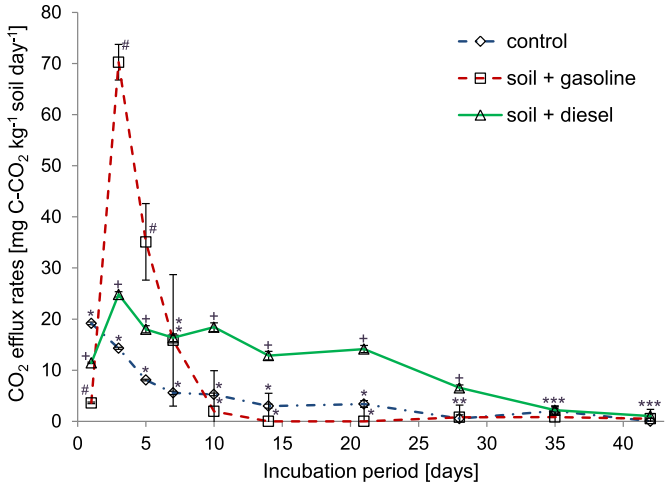

Fig. 2. CO_2 efflux rates from soil contaminated with petroleum products. Whiskers show standard errors of the means (n = 3). For each time point, treatments with the same symbol are not significantly different ($p < 0.05$).

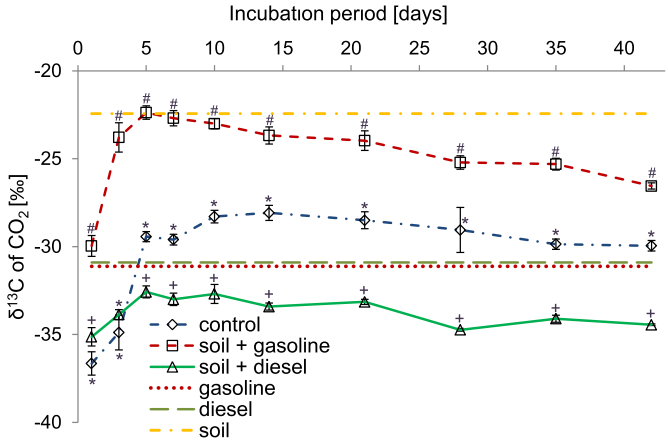

Fig. 3. $\delta^{13}C$ of CO_2 released from soil with and without addition of petroleum products [15]. Whiskers show standard errors of the means (n = 3). Symbols indicate significant differences between treatments on each sampling day ($p < 0.05$).

increase of $\delta^{13}C$–CO_2: from about −30‰ to more than −22.4‰ on the 5th day. Then $\delta^{13}C$ slowly decreased and reached −26.6‰ at the end of the experiment (42nd day). Contamination by diesel also caused an increase of $\delta^{13}C$-CO_2, but the intensity of the change was much less. The $\delta^{13}C$ decrease after the 5th day of the incubation was also evident (−34.5‰ at the end of the experiment).

Using the $\delta^{13}C$ values for CO_2 partitioning requires correctly considering the ^{13}C fractionation by decomposition. To assess the ^{13}C fractionation by decomposition of SOM, we can use the $\delta^{13}C$ values for CO_2 released from soil without added petroleum products (Fig. 3). This ^{13}C fractionation, as the difference between a substrate ($\delta^{13}C^{soil}$) and a product ($\delta^{13}C^{CO2\text{-}soil}$), was calculated for the whole incubation period. This ^{13}C fractionation was +14.2‰ at the beginning and stabilized at +7.5‰ at the end of incubation.

To consider the ^{13}C fractionation in petroleum decomposition, additional incubations in a substrate with very low C content are necessary. Gasoline and diesel were therefore incubated in sand with an inoculum of soil microorganisms. The $\delta^{13}C$ values of released CO_2 differed between the two products (Fig. 4), and clearly showed opposing ^{13}C fractionations. The ^{13}C fractionation for diesel mineralization increased from +0.1‰ to +4.0‰ (values for the 1st

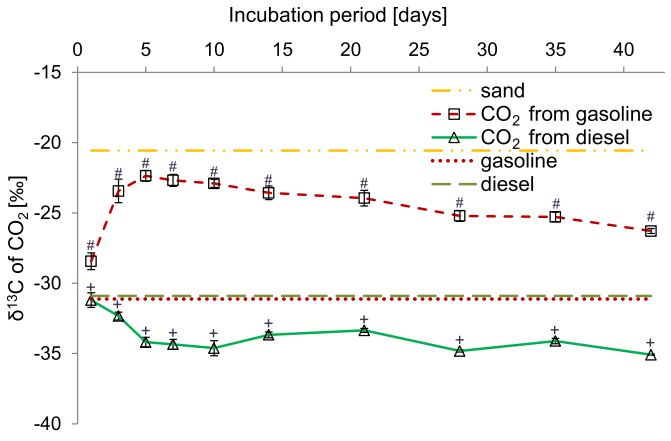

Fig. 4. $\delta^{13}C$ values of CO_2 released by decomposition of petroleum products in sand inoculated with soil microorganisms. The $\delta^{13}C$ values for gasoline and diesel reflect the dynamics of ^{13}C fractionation during decomposition. Whiskers show standard errors of the means (n = 3). Symbols indicate significant differences between treatments on each sampling day ($p < 0.05$).

and 42nd day of incubation, respectively). The respective values for gasoline were negative: from −8.8‰ to −2.7‰, whereby the extreme negative value occurred at the 5th day of incubation.

These ^{13}C fractionation values as well as the $\delta^{13}C$ dynamics clearly show that the $\delta^{13}C$ of the substrates (diesel and gasoline) cannot be directly used to calculate CO_2 partitioning. The $\delta^{13}C$ of CO_2 released from individual sources must be considered. Furthermore, the dynamics of $\delta^{13}C$ in CO_2 show that ^{13}C-depleted petroleum compounds in diesel are decomposed within 42 days, whereas mainly ^{13}C-enriched substances are involved in gasoline mineralization.

Combining IRMS with the analysis of CO_2 concentration enables determining which fraction of the total CO_2 released from the soil comes from the native SOM and which fraction from the added substrate [23]. Thus, using Eq. (8), these fractions were calculated (Fig. 5).

Adding gasoline and diesel strongly decreased the mineralization of native SOM (Fig. 5). This suppression was especially strong after gasoline addition, leading to a >95% decrease of SOM mineralization. This slowdown is from about 150 mg C–CO_2 kg^{-1} of dry soil (value obtained for pure soil, i.e. control) to about 6 mg and

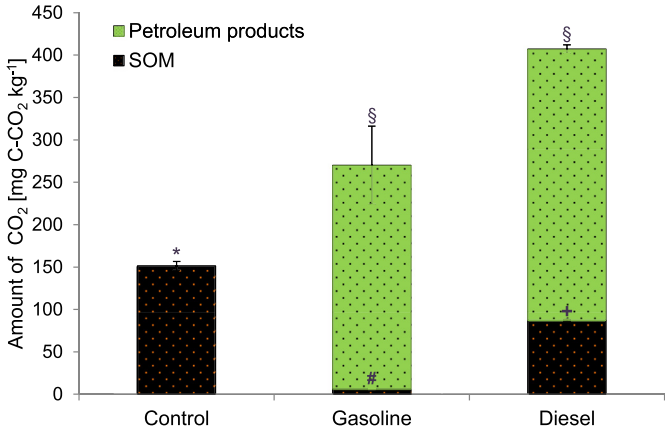

Fig. 5. CO_2 released from the soil during 42 days partitioned for two C sources: petroleum products and SOM. Symbols indicate significantly different contributions by the respective source between the treatments. Symbols indicate a significant difference in the amount of CO_2 from that source, when compared between treatments.

87 mg C–CO_2 kg^{-1} soil for gasoline and diesel, respectively. Consequently, gasoline was much more toxic for microorganisms decomposing SOM than diesel.

The dynamics of inhibition of SOM mineralization were different for both petroleum products, as reflected by the fraction of CO_2 resulting from mineralization (Fig. 6 B). The contamination by gasoline almost completely blocked SOM degradation in the soil (dashed line in Fig. 6 B). The residual activity of microorganisms decomposing SOM was measurable only up to day 5. Thereafter, the microbial activity nearly ceased. It corresponds to the degradation of gasoline, which strongly increased in the first days of incubation, peaking (about 68 mg C–CO_2 kg^{-1} day^{-1}) on day 3 (dashed line in Fig. 6 A).

A different CO_2 trend was observed when the soil was contaminated with diesel. The SOM was degraded by day 28 (solid line in Fig. 6 B). The $\delta^{13}C$ of CO_2 emitted from the diesel-contaminated soil during the first four days of the experiment was close to the control soil value (Fig. 3). Accordingly, microorganisms most intensively decompose the native carbon in soil samples with diesel during the first 28 days (Fig. 6 B). Nonetheless, the degradation dynamics peaked on the 3rd day – about 15 mg C–CO_2 kg^{-1} day^{-1}. Diesel degradation continued during the whole experiment (Fig. 6 A).

Soil contamination by gasoline and diesel increased the total CO_2 efflux. However, nearly all the produced CO_2 derived from the petroleum products, which were mineralized to 2.4% and 2.9% for gasoline and for diesel, respectively. Therefore, we assume that only very easily decomposable components of petroleum products were mineralized during 42 days. Adding petroleum products strongly decreased the mineralization of native SOM.

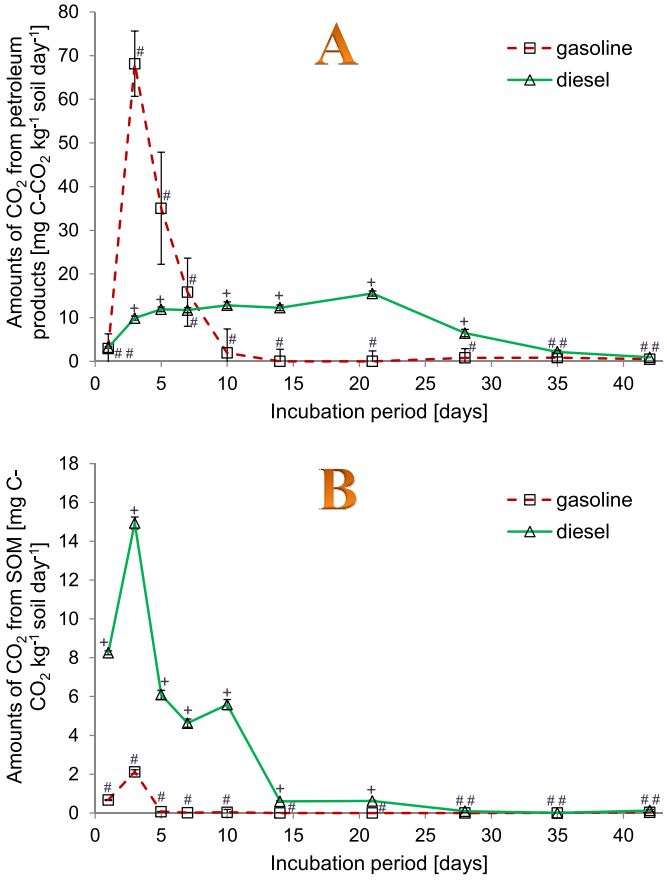

Fig. 6. CO_2 derived from degradation of petroleum products (Fig. 6 A) and SOM (Fig. 6 B). Whiskers show standard errors of the means (n = 3). Symbols indicate significant differences between treatments on each sampling day ($p < 0.05$).

4. Discussion

4.1. CO_2 fluxes

Adding petroleum products to the soil strongly affected microbial activities. The microorganisms started to decompose the added C of petroleum products, consequently increasing CO_2 efflux from the soil. During the whole experiment, the CO_2 efflux dynamics differed significantly in the control versus contaminated soil. Contamination by gasoline and diesel led to high CO_2 efflux in the first days, with the peak on day 3. The maximum CO_2 production in the control soil was reached within one day. This shift in post-contamination peaks indicates that the microorganisms decamping SOM were strongly depressed, but the microorganisms responsible for petroleum product mineralization needed about 3 days to establish and become active. A similar shift (4 days) in CO_2 efflux from soil contaminated with crude oil was reported by Zyakun et al. [24]. However, the CO_2 efflux reported there was longer and lasted throughout the 67 days of incubation (comparing to the 42 days here for diesel – Fig. 2). This difference may reflect the use of different soils and the actual chemical composition of the petroleum products [25,26]. The differences in CO_2 release from gasoline- and diesel-contaminated soil were caused by the substrate specifics (Figs. 1 and 2). The gasoline contains much lighter hydrocarbons, which are more easily decomposed by microorganisms [27].

Adding petroleum products inhibited the decomposition of native SOM to a large extent in the case of diesel and almost completely in gasoline (Fig. 5). Microbial activity was stopped at the moment of contamination (Figs. 1 and 2). Thereafter, CO_2 efflux on the first day in both contaminated samples was much lower than in the control. Importantly, the activity of native microorganisms depended on the petroleum product (Figs. 1, 2 and 5). The native microorganisms remained active when the diesel was added, but were completely inactivated by gasoline contamination. This difference can be explained by the different volatilities of the products [28]. Gasoline is much more volatile and thus penetrated the soil pores much more easily and faster, stopping microbial activity more abruptly and more strongly. The much less volatile diesel penetrated soil pores slowly and did not enter the fine pores. Consequently, microbial activity did not cease completely before the new microbial groups started to decompose the diesel, and the native OM continued to be mineralized (Fig. 6).

4.2. Isotopic signature of CO_2 and decomposition rates of petroleum products

As noted above, microorganisms preferentially decompose the lighter hydrocarbons (one reason for the differences in CO_2 release from soil). This phenomenon is also crucial in explaining the isotope ratios in CO_2 emitted from contaminated soils.

The analysis of the $\delta^{13}C$ of CO_2 in the first days shows that the increase of $\delta^{13}C$ in the gasoline treatment was very rapid. The value was much higher not only compared with the control soil, but it even exceeded the $\delta^{13}C$ of CO_2 from pure gasoline (Fig. 3). The increase above the value of pure gasoline reflects the selective consumption of the individual gasoline components. This process is common for mixed substrates and is frequently termed 'preferential substrate utilization' [29]. The $\delta^{13}C$ of −31.12‰ is the averaged value of all hydrocarbons and other substances present in the gasoline mixture. These mixture components have different $\delta^{13}C$ values ranging from −24.71‰ to −32.90‰ for alkanes and

from −21.74‰ to −28.33‰ for BTEX components; thermogenic gases, in contrast, range from −15 to −50‰ [30–33]. Because of the different decomposition rates of individual ingredients by microorganisms, the $\delta^{13}C$ of CO_2 emitted from the soil can exceed that of gasoline.

The $\delta^{13}C$ value of pure diesel was close to that of pure gasoline. However, the $\delta^{13}C$ of CO_2 emitted from diesel-contaminated soils was lower than for pure diesel. Additionally, in the first three days, these values were similar to those of the control. This can reflect not only the selective consumption of individual components in diesel but also significant microbial consumption of C from the native organic matter during the first 3 days (Fig. 6 B).

Isotopic fractionation during mineralization of native soil organic matter (Fig. 3) showed that microorganisms decompose compounds depleted in the heavier carbon (^{13}C), similar as in contamination with diesel. This stands in contrast to gasoline: microorganisms decompose ^{13}C-enriched compounds. The enrichment of ^{13}C can be explained as follows. Many studies showed that lighter hydrocarbons have higher $\delta^{13}C$ values compared to heavier hydrocarbons [34–36]. Accordingly, the $\delta^{13}C$ values decrease with increasing chain length [37]. Gasoline contamination of soil introduces lighter hydrocarbons ($C_3 - C_{12}$) [31]. Microorganisms prefer these hydrocarbons over heavier ones. Typically, the rate and/or intensity of hydrocarbon degradation proceeds in the following order: linear alkanes > branched alkanes > cycloalkanes/aromatic hydrocarbons (with one or two rings) > polyaromatic hydrocarbons (PAH; with more than two rings) [38]. The factor which could inhibit the consumption of native carbon in the soil was the presence of lighter hydrocarbons. Therefore, noting that gasoline was practically the only substrate for microbial activity (Figs. 5 and 6 A) and that the lighter gasoline components are preferred by microorganisms, the decomposition should lead to higher $\delta^{13}C$ values compared to the whole petroleum products. Adding heavier hydrocarbons to the soil (i.e. diesel contamination) leads to the co-degradation of both carbon sources: SOM and the hydrocarbons. This co-degradation took place because heavier hydrocarbons are less attractive for microorganisms than lighter ones (Fig. 6).

5. Conclusions

The contamination of soil by petroleum products negatively affected microorganisms and strongly decreased their functions, e.g. SOM decomposition. Soil microorganisms needed 1–3 days to start to decompose the petroleum products. The decomposition dynamics, however, differed between gasoline and diesel. The easily degradable compounds in the former were decomposed within the first 10 days, whereas the decomposition of the easily degradable diesel products lasted about 5 weeks.

Gasoline as a lighter hydrocarbon is more volatile and penetrated more easily into soil pores than diesel. Therefore, the degradation of native SOM nearly ceased after adding gasoline as compared to heavier hydrocarbons such as diesel.

The analysis of the $\delta^{13}C$ of CO_2 from sand with petroleum products showed strong ^{13}C isotopic fractionation, differing for diesel and gasoline. At the beginning of incubation, the ^{13}C-depleted petroleum compounds of diesel were decomposed, whereas ^{13}C-enriched CO_2 was released by decomposition of easily decomposable gasoline compounds. This ^{13}C fractionation should be considered by partitioning the CO_2 fluxes from soil after petroleum products contamination in order to correctly evaluate the effects of petroleum contamination on SOM decomposition.

Acknowledgements

We thank the Catholic Academic Exchange Service (KAAD) for the scholarship award for Wioleta Stelmach.

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