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Radiation-induced impacts on the degradation of 2,4-D and the microbial population in soil microcosms

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ABSTRACT

In a soil microcosm experiment, the influence of low-level ^{137}Cs and ^{90}Sr contamination on the degradation of ^{14}C-ring-labeled 2,4-dichlorophenoxyacetic acid (2,4-D) was studied. Two differently treated soils (one native soil and one soil sterilized and reinoculated with a biotic soil aliquot) were artificially contaminated with various concentrations of ^{137}Cs and ^{90}Sr as nitrate salts. The cumulative doses increased up to 4 Gy for 30 days of incubation in soil microcosms. Changes in microbial community structure were observed with help of the denaturing gradient gel electrophoresis (DGGE). A radiation-induced impact appeared only in the microcosms treated with 30 times the maximum contamination appearing in the exclusion zone around reactor 4 in Chernobyl. In contrast to the less contaminated soils, the mineralization of 2,4-D was delayed for 4 days before it recovered.

Slight shifts in the microbial communities could be traced to radiation effects. However, other parameters had a major impact on mineralization and community structure. Thus the sterilization and reinoculation and, of course, application of the 2,4-D were predominantly reflected in the $^{14}CO_2$ emissions and the DGGE gel patterns.

1. Introduction

The explosion of reactor 4 in the nuclear power plant near Chernobyl in 1986 raised public awareness of the usage of nuclear power in Western Europe. However, in Eastern Europe it was necessary to evacuate the population and to create an exclusion zone around the reactor. The accident also affected agriculture. As a consequence of the deposition of radioactive fall-out large quantities of crops and cow milk had to be destroyed, even in Western Europe. Besides fuel particles, the most important radionuclides deposited were several isotopes of iodine (amongst others ^{131}I and ^{129}I), ^{137}Cs, ^{134}Cs and ^{90}Sr (UNSCEAR, 2000). Iodine-131 has a half-life of only 8 days, but it is responsible for the increased occurrence of thyroid diseases as a consequence of the accident (Brenner et al., 2011). Today the emitted radioactive iodine, except ^{129}I, no longer has any effect due to physical decay. However, due to the long half-life and the chemical similarity to potassium and calcium, the residence time of ^{137}Cs and ^{90}Sr in the upper soil can amount to several decades (Gastberger et al., 2000; Rafferty et al., 2000; Konopleva et al., 2009). Due to the large input of nutrients, organic matter, warmer temperatures and the sufficient exchange of gases (such as carbon dioxide and oxygen) in upper soil layers, the amount and activity of microorganisms is high. As yet, it is not clear how radionuclides in soil affect the microbial activity, e.g. the degradation of agricultural chemicals. From irradiation studies with high dose rates up to several kGy (Gy, Gray: absorbed dose in J kg^{-1}) it is known that the higher the radioactivity the lower the ability of microorganisms to metabolize complex organic compounds (McNamara et al., 2007). However, the naturally occurring doses and even the dose rates caused by accidents in nuclear facilities are much lower, so an extrapolation to realistic conditions is quite difficult.

In this study, we investigated the effects of ^{137}Cs and ^{90}Sr, respectively, on the mineralization of the ^{14}C-ring-labeled herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). 2,4-D is one of the herbicides most frequently used for broad-leaved weeds worldwide, it is the chlorinated form of a natural plant hormone, an auxin (Lerch et al., 2007). 2,4-D is well suited as a substrate and carbon source for microorganisms since it consists of the easily available acetic side chain and the more recalcitrant phenyl ring.

The microcosms were artificially contaminated with ^{137}Cs and ^{90}Sr corresponding to the radioactivity caused by ^{137}Cs in the

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exclusion zone around reactor 4 in Chernobyl. The formal threshold for the exclusion zone is 1.48 MBq m^{-2} (Bq: Bequerel, decay s^{-1}), which is transferred to soil mass as 11.4 Bq g^{-1} (assumed soil parameters: soil density: 1.3 g cm^{-3}, depth of homogeneous radionuclide distribution: 0.1 m). The lowest artificial contaminations were in the range from 20 to 50 Bq g^{-1}. Hotspots in the exclusion zone exhibit up to 40 MBq m^{-2}, which was considered in the experiment by the medium contamination ranging from 490 to 980 Bq g^{-1}. The highest radionuclide treatments were between 8150 Bq g^{-1} (^{137}Cs) and 9610 Bq g^{-1} (^{90}Sr) (Table 1).

The aim of this study was to investigate whether radioactive contamination with ^{137}Cs and ^{90}Sr corresponding to the Chernobyl exclusion zone has an impact on (i) the degradation of the agriculturally used herbicide 2,4-D and (ii) the bacterial and fungal community structure in agricultural soils incubated in microcosms.

2. Materials and methods

2.1. Soil sampling and sample preparation

Soil samples were collected randomly from the plough layer (5–20 cm) at the field site Merzenhausen near Forschungszentrum Jülich. In previous years, the field had been cultivated alternately with barley, rape and winter wheat. The soil was characterized by a sand, silt and clay content of 3, 79 and 18%, respectively. The pH was 7 (measured in 0.01 M $CaCl_2$), cation exchange capacity (determined from exchange with NH_4Cl solution) is 12.0 cmol kg^{-1} and C_{org} was 1.04% (Kasteel et al., 2005). Coarse organic components and roots were removed and the field-moist soil was sieved ($\leq$2 mm) and mixed thoroughly. To obtain more significant results in the analysis of the community structure, in half of the microcosms the soil was sterilized and reinoculated with 5% biotic bulk soil. The reproductive rates of microorganisms in soil are more sensitive to environmental changes than metabolic or death rates (Morris and Blackwood, 2007), thus a larger radiation impact on freshly sterilized and reinoculated coommunities was expected, resulting in strong disparities in the DGGE gel patterns. Fungal spores were eliminated by autoclaving three times in succession with a mean time of 24 h.

2.2. Application of 2,4-D and radionuclides

Prior to application, the ^{14}C-ring-labeled 2,4-D (American Radiolabeled Chemicals, Inc., St. Louis, Missouri, USA, specific activity 2035 GBq mol^{-1}) was diluted with non-radioactive analytical grade 2,4-D (Sigma–Aldrich, St. Louis, Missouri, USA). After dissolution in ethanol (100 μg mL^{-1}), the 2,4-D was applied to soil aliquots (5 g dry weight) which had previously been ashed at 600 °C for 24 h. The combustion removed hydrophobic humic substances to allow better absorption. Immediately after 2,4-D application, ^{137}Cs and ^{90}Sr were applied as aqueous solutions (concentration depending on the final radioactivity between 2 kBq mL^{-1} and 1000 kBq mL^{-1}). After drying, the aliquots were added to the soils and mixed thoroughly in an end-over-end shaker for 45 min. The final concentration of 2,4-D was 100 μg g^{-1}, ^{14}C activity was 101 Bq g^{-1}. The radioactivity and the dose rates of ^{137}Cs and ^{90}Sr can be seen in Table 1. The cumulative dose rates for 30 days of incubation ranged from 5.2×10^{-3} Gy to 4.39 Gy. The ^{137}Cs and ^{90}Sr were purchased as nitrate salts from Ritverc, St. Petersburg, Russia.

The radioactive contamination of the soils was determined in the Central Division of Research Reactors and Nuclear Service at Forschungszentrum Jülich. The absorbed dose [Gy; gray] was calculated by the Central Department of Radiation Protection at Forschungszentrum Jülich by using a particle track calculation and a Monte Carlo simulation (MCNP5, Los Alamos National Laboratory).

2.3. Microcosm setup

The microcosms were performed in triplicate. The microcosms consisted of 500 mL lab bottles filled with 100 g soil dry weight. The $^{14}CO_2$ respired from the 2,4-D mineralization was adsorbed in 1 mL, 1.5 M sodium hydroxide. The CO_2 traps were replaced each other day, filled with 3 mL deionized water and 10 mL scintillation cocktail (Insta-Gel Plus, Perkin–Elmer, Waltham Massachusetts, USA) and measured with a liquid scintillation counter (LSC, Liquid Scintillation Analyzer 2500 TR, TriCarb, Packard). The cumulative values were calculated by the stepwise addition of the mineralization rates measured each second day to the rates from the previous days. The mineralization rate is defined by the measured $^{14}CO_2$ per elapsed time period divided by the totally applied ^{14}C radioactivity as 2,4-D (data not shown). While changing the CO_2 traps the microcosms were flushed with fresh air from a peristaltic pump. Soil moisture was set to 50% of water-holding capacity (WHC) (OECD, 2002; Rocha et al., 2006) with purified water (MilliQ, Millipore). Water loss was controlled weekly and water was added as necessary. The microcosms were incubated for 30 days at 20 ± 1 °C.

2.4. Denaturing gradient gel electrophoresis

DNA was extracted with the extraction kit "FastDNA Spin Kit for Soil, BIO101" from Qbiogene, Carlsbad, USA. The DNA was amplified with the Thermocycler iCycler IQ by Bio Rad Laboratories, USA. In the case of the 16S rDNA DGGE, the primers L1401 (5′- CGG TGT GTA CAA GAC CC -3′) and U968GC (5′- CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA CCTTAC -3′) were chosen. In the case of 18S rDNA DGGE, we used the primers NS1 (5′-GTA GTC ATA TGC TTG TCT C-3′) and GCFung (CGC CCG CCG CGC

Table 1
Radioactivity (in Bq g^{-1}) and dose rate (in Gy h^{-1} and Gy 30 d^{-1}) applied to the soil microcosms. Control soils do not contain ^{137}Cs or ^{90}Sr. Radioactivity measured in triplicate (±mean standard deviation of $n = 3$).

	Native			Sterilized and reinoculated		
	Bq g^{-1}	Gy h^{-1}	Gy 30 d^{-1}	Bq g^{-1}	Gy h^{-1}	Gy 30 d^{-1}
^{90}Sr	20 ± 1.6	1.4×10^{-5}	0.01	20 ± 2.4	1.4×10^{-5}	0.01
^{90}Sr	500 ± 28	3.6×10^{-4}	0.26	490 ± 40	3.5×10^{-4}	0.25
^{90}Sr	8150 ± 250	5.9×10^{-3}	4.25	8410 ± 553	6.1×10^{-3}	4.39
^{137}Cs	50 ± 1.9	9.0×10^{-6}	0.01	40 ± 1.4	7.2×10^{-6}	0.01
^{137}Cs	940 ± 21	1.7×10^{-4}	0.12	980 ± 31	1.8×10^{-4}	0.13
^{137}Cs	9610 ± 264	1.7×10^{-3}	1.22	9410 ± 866	1.7×10^{-3}	1.22
Control	0	0	0	0	0	0