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Effects of low-level radioactive soil contamination and sterilization on the degradation of radiolabeled wheat straw

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ABSTRACT

After the explosion of reactor 4 in the nuclear power plant near Chernobyl, huge agricultural areas became contaminated with radionuclides. In this study, we want to elucidate whether ^{137}Cs and ^{90}Sr affect microorganisms and their community structure and functions in agricultural soil. For this purpose, the mineralization of radiolabeled wheat straw was examined in lab-scale microcosms. Native soils and autoclaved and reinoculated soils were incubated for 70 days at 20 °C. After incubation, the microbial community structure was compared via 16S and 18S rDNA denaturing gradient gel electrophoresis (DGGE). The radioactive contamination with ^{137}Cs and ^{90}Sr was found to have little effect on community structure and no effect on the straw mineralization. The autoclaving and reinoculation of soil had a strong influence on the mineralization and the community structure. Additionally we analyzed the effect of soil treatment on mineralization and community composition. It can be concluded that other environmental factors (such as changing content of dissolved organic carbon) are much stronger regulating factors in the mineralization of wheat straw and that low-level radiation only plays a minor role.

1. Introduction

The explosion of reactor 4 in Chernobyl on 26 April 1986 and recent activities in Japan have shown that the usage of nuclear power involves large-scale risks. While the ecological implications for Europe have not been so immense to date, the landscape around the reactors became highly contaminated. Cultivating the land and living in these areas may cause health risks to the human population (Dederichs et al., 2009).

Within the framework of post-Chernobyl programs, the pathways of radionuclides from soil to plants and into the human food chain were thoroughly examined (Bilo et al., 1993; Nisbet and Woodman, 2000). However, the effects of radioactive contamination on the soil micro-flora and its functions still remain widely unknown. Numerous studies observed the radiation effects on microorganisms but most of these studies used high radiation doses of up to several kGy or observed the effects on single microbial strains (McNamara et al., 2007; Pitonzo et al., 1999b). Due to these experiments, which usually involved sterilization of the soils, the lethal doses for several isolated strains are well known. Gamma-irradiation doses around 10 kGy are referred to as sub-sterilizing doses. This level of radiation has a lethal effect on most of the actinomycetes, fungi and invertebrates. The majority of bacteria is eliminated at 20 kGy and doses higher than 70 kGy kill radio-resistant bacteria (McNamara et al., 2003). Only a few studies have observed microbial behavior under low-level irradiation, e.g. Gochenaur and Woodwell (1974) and Jones et al. (2004). To our knowledge, studies conducted with contamination such as in the Chernobyl zone have not been performed to date. The radioactive contamination in agricultural fields around Chernobyl ranging from 400 to 20,000 kBq m^{-2} may play an important role from an ecological point of view, since such levels of contamination are more likely to appear and they affect larger areas than contaminations in the range of several kGy. In the case of Chernobyl, more than 300 km^2 were contaminated with ^{90}Sr at a level higher than 400 kBq m^{-2} (Kashparov et al., 2001). Large parts of these areas had been used agriculturally, and today smaller parts are in use again by remigrated people (Dederichs et al., 2009).

Microbial communities have the capability to influence various ecosystem properties. Microorganisms are responsible for organic matter mineralization, humus formation in soil and the recycling of nutrients. Due to the capability of soil microbes to degrade numerous pollutants, they furthermore play an important role in protecting groundwater and improving soil quality. Since

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remediation of such huge areas is extremely expensive and the natural migration of several radionuclides is low (Konopleva et al., 2009; Thiry and Myttenaere, 1993; Zhu and Shaw, 2000), it is important to know how radionuclide contamination affects the soil micro-flora, as this in turn impacts directly on the soil quality.

In our study, we focused on the effects of soil contamination with ^{137}Cs and ^{90}Sr on the bacterial and fungal community structure and the degradation of wheat straw. The amount of radioactive contamination in soil was based on contamination in the zone around Chernobyl. The formal threshold for the exclusion zone is 1.48 MBq m^{-2} (40 Ci km^{-2}) for ^{137}Cs. Transferred to soil mass this is 11.4 Bq g^{-1} (assumed parameters: soil density: 1.3 g cm^{-3}, soil depth of homogeneous radionuclide distribution: 0.1 m). The highest values for ^{137}Cs in the 10 km zone around the reactor in Chernobyl are around 40 MBq m^{-2}, which conforms to 307 Bq g^{-1} (personal communication with Mr. Valery Kashparov, 08.12.2009) when equal soil parameters were assumed. Similar values up to 629 Bq g^{-1} were reported by Romanovskaya et al. (1998). The artificial contamination in the microcosms set up for our experiment began at 692 Bq g^{-1} and ranged up to 15,033 Bq g^{-1}.

The objectives of this study were: i) to observe the impact of low-level radioactive contamination on the degradation of ^{14}C-labeled wheat straw in soil and ii) to evaluate potential microbial community alteration caused by the applied radioactivity.

2. Material and methods

2.1. Experimental soil

The study was performed with an orthic luvisol field soil from Merzenhausen, Germany. The underlying sediments were quaternary sediments, mostly consisting of fluvial deposits from the Rhine/Maas River and the RurRiver system, covered by Pleistocene and Holocene eolian sediments. The used soil from the ploughing layer (0–0.35 m) consisted of 3% sand, 79% silt, and 18% clay. The pH was 7.0 (measured in 0.01 M $CaCl_2$, the cation exchange capacity 12.0 cmol kg^{-1} (determined from exchange with a NH_4Cl solution), and the organic carbon content was 1.04% (data from Kasteel et al. (2005)).

2.2. Soil sampling and sample preparation

Soil samples were taken from the plough layer (5–20 cm) at the Merzenhausen field site, since the maximum microbial activity (Jones et al., 2004) and radionuclide contamination is expected in the upper layer (Delvaux et al., 1996; Drissner et al., 1998; Rafferty et al., 2000). The field moist soil was sieved ($\leq$2 mm) and thoroughly homogenized. Coarse organic components and roots were removed manually. Until the experimental radionuclide application, the soil was stored at 4 °C in open PE bags, to allow aeration of the soil samples.

Since the reproductive rates of microorganisms in soil are more sensitive to environmental changes than the metabolic or death rates (Morris and Blackwood, 2007), the soil was sterilized in half of the microcosms and subsequently reinoculated with 1% (of total soil mass) biotic, non-sterilized soil. To eliminate fungal spores, the sterilization was performed by autoclaving three successive times with a meantime of 24 h. The sterilized soil was dried at 105 °C for 24 h. The aim of the sterilization and reinoculation was to intensify microbial growth in the reinoculated microcosms compared to the non-sterilized microcosms. We expected to obtain more significant results in our analysis of the microbial population development, and thus more significant disparities in the DGGE patterns. Indeed, it is well known that harsh treatment such as sterilization affects chemical and physical soil conditions. The amount of water-extractable carbon and nitrogen increases and soil aggregates are destroyed (Berns et al., 2008; McNamara et al., 2003). Furthermore, microorganisms killed during the sterilization process deliver more available nutrients and dissolved organic carbon (DOC) (Marschner and Bredow, 2002). The DOC and total nitrogen content (TN) were determined via water extraction. Field moist soil and sterilized soil, respectively, (20 g according to dry soil mass) were suspended in deionized water (MilliQ) in the ratio of 1:8 (m/m). The mixture was shaken for 12 h (150 rpm) and centrifuged for 90 min (10,000× g). The supernatant was filtrated with 0.45 μm membrane filter and analyzed with TOC-V CPH Total Organic Carbon Analyzer (Shimadzu Scientific Instruments, Japan). All chemicals used for application were of analytical grade.

2.3. Application of straw and radionuclides

Both radionuclides ^{137}Cs and ^{90}Sr were delivered as nitrate salts (Ritverc, St. Petersburg). After dissolving in deionized water, the radionuclides were applied to small amounts of soil (5 g) which were ashed at 600 °C for 24 h. Previous ashing was applied to remove hydrophobic humic substances to allow absorption of water without lump formation. After drying, the soil aliquots were grounded and added to the soil microcosms.

The applied wheat straw (cultivar: Taifun) was produced in 2005 at the Institute of Bio- and Geosciences (IBG-3), Forschungszentrum Jülich. The specific activity was 53.8 ± 0.8 kBq g^{-1} ($n = 5$). Due to this high activity, the straw was diluted with non-radioactively labeled wheat straw. The final specific radioactivity was 8.8 ± 0.2 kBq g^{-1} ($n = 5$). The straw contained 39.5% C and 1.24% N. Before it was added to the soil, the straw was ground to less than 500 μm with an ultra-centrifugal mill at 15,000 rpm (Retsch, Germany). The soil-straw mixture was thoroughly mixed in an end-over-end shaker for 45min. The radioactivity and dose rates were determined by the Central Division Research Reactors and Nuclear Service at Forschungszentrum Jülich. The absorbed dose [Gy; Gray] was calculated using a particle tracks calculation and a Monte Carlo simulation (MCNP5, Los Alamos National Laboratory). The applied straw accounted for 2% (w/w) of soil dry mass, which is much higher than usual in agricultural field sites. This higher straw addition aimed to stimulate an enhanced growth of the microbial soil population.

2.4. Microcosm setup

Each microcosm experiment was performed in triplicate. As microcosms, 500 ml lab bottles were filled with 100 g soil (dry weight, including 2 g wheat straw). The water content was set to 50% of the soil's water holding capacity (WHC) (OECD, 2002; Rocha et al., 2006) using purified water (MilliQ, Millipore). The bottles were tightly closed and mineralization was determined by measuring the evolved $^{14}CO_2$. Radioactive carbon dioxide was trapped in 2 ml 2 M sodium hydroxide solution in a glass vial inside the microcosm. This was replaced every second day. The samples were mixed with 8 ml deionized water and 10 ml scintillation cocktail (Insta-Gel Plus, Perkin–Elmer) and measured for 15 min, using a Liquid Scintillation Analyzer 2500 TR, TriCarb, Packard. When the carbon dioxide traps were changed, the bottles were also flushed with fresh air from a peristaltic pump. A constant ratio between the adsorbed and measured $^{14}CO_2$ and the complete CO_2 released from mineralization over the incubation time is postulated. So the measured $^{14}CO_2$ divided by the totally applied ^{14}C radioactivity as wheat straw represents the mineralization rate. The percentage of the cumulative $^{14}CO_2$ evolved is calculated by adding up the ^{14}C radioactivity measured by the LSC with the measured values from the days before. The last value of the evolved $^{14}CO_2$ represents the total activity adsorbed in NaOH as carbon dioxide