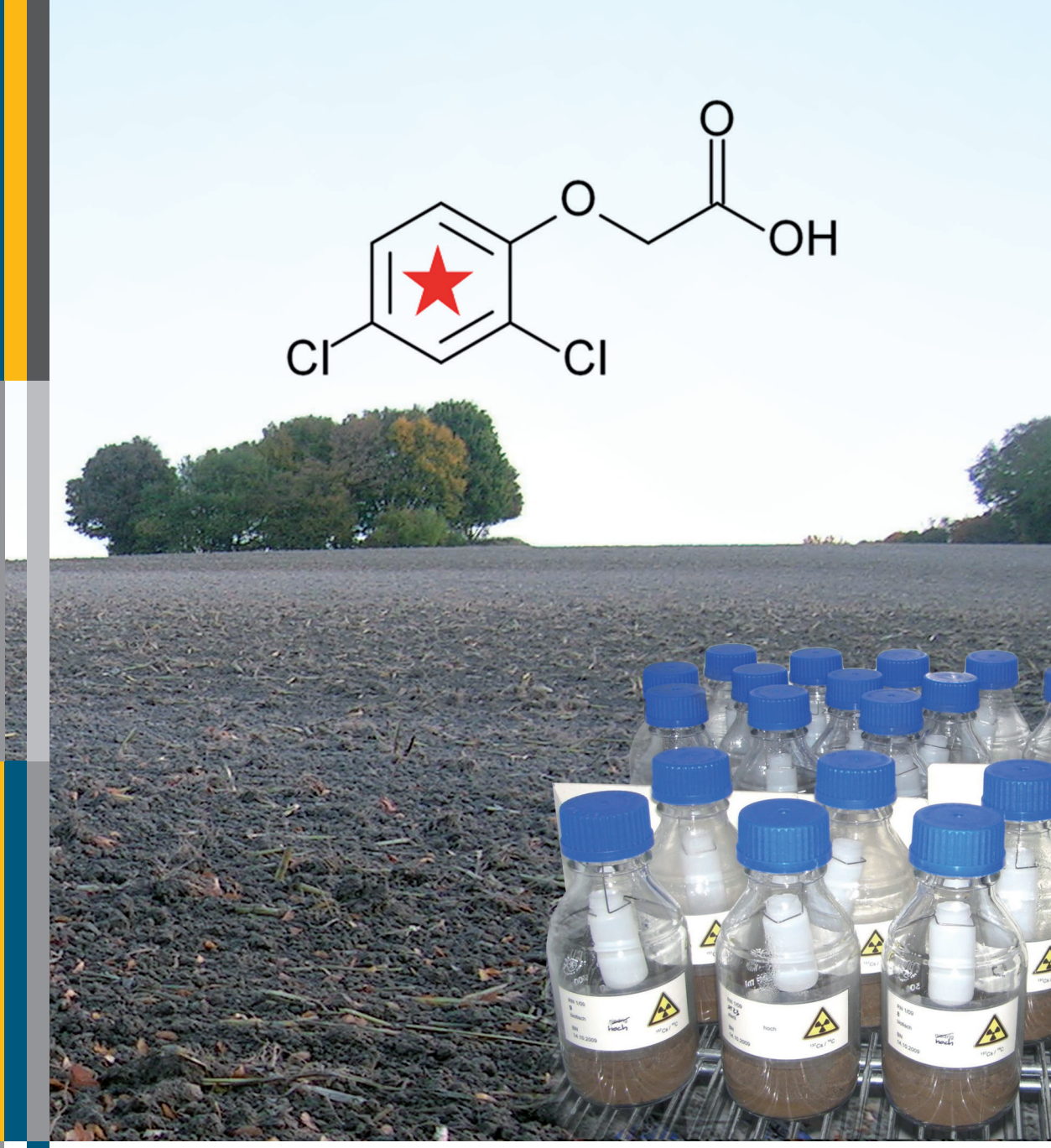


Effects of ^{137}Cs and ^{90}Sr on structure and functional aspects of the microflora in agricultural used soils

Bastian Niedrée

Mitglied der Helmholtz-Gemeinschaft

JÜLICH
FORSCHUNGSZENTRUM

Schriften des Forschungszentrums Jülich
Reihe Energie & Umwelt / Energy & Environment Band / Volume 162

Forschungszentrum Jülich GmbH
Institute for Bio- and Geosciences (IBG)
Agrosphere (IBG-3)

Effects of ^{137}Cs and ^{90}Sr on structure and functional aspects of the microflora in agricultural used soils

Bastian Niedrée

Schriften des Forschungszentrums Jülich
Reihe Energie & Umwelt / Energy & Environment Band / Volume 162

ISSN 1866-1793 ISBN 978-3-89336-843-3

Bibliographic information published by the Deutsche Nationalbibliothek.
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available in the Internet at http://dnb.d-nb.de.

Publisher and Distributor:	Forschungszentrum Jülich GmbH Zentralbibliothek 52425 Jülich Phone +49 (0) 24 61 61-53 68 · Fax +49 (0) 24 61 61-61 03 e-mail: zb-publikation@fz-juelich.de Internet: http://www.fz-juelich.de/zb
Cover Design:	Grafische Medien, Forschungszentrum Jülich GmbH
Printer:	Grafische Medien, Forschungszentrum Jülich GmbH
Copyright:	Forschungszentrum Jülich 2013

Schriften des Forschungszentrums Jülich
Reihe Energie & Umwelt / Energy & Environment Band / Volume 162

D 5 (Diss., Bonn, Univ., 2012)

ISSN 1866-1793
ISBN 978-3-89336-843-3

Abstract

At long sight ^{137}Cs and ^{90}Sr are the main radionuclides responsible for the contamination of agricultural soils due to core melts in nuclear power plants such as Chernobyl or Fukushima. Once deposited on the soil surface, the two radionuclides remain in the upper soil layer for several decades. In the upper soil layer the highest microbial activity can be found, due to high organic matter contents, warm temperatures and gas exchange with the atmosphere. Hence, in contaminated soils microorganisms in upper soil layers (e.g. the plow layer on agricultural fields) are exceedingly exposed to radioactivity. However, no data are available how radioactive contaminations with ^{137}Cs or ^{90}Sr in a realistic order of magnitude affect the microbial community and its functions in soils.

This dissertation discusses the effects of radioactive contaminations on the microbial community structure and some of its functions in soils. Therefore, typical agricultural soils, an *Orthic Luvisol* from field site Merzenhausen and a *Gleyic Cambisol* from field site Kaldenkirchen-Hülst were artificially contaminated with various concentrations of ^{137}Cs and ^{90}Sr and partly applied with radiolabeled substrates and incubated in soil microcosms under controlled laboratory conditions. The lower radionuclide concentrations corresponded to the contaminations in the Chernobyl exclusion zone, the higher concentrations were up to 50-fold that of the maximum occurring hotspots (^{137}Cs) in this zone. In three experiments the effects of the ionizing radiation on the bacterial and the fungal community structure (16S and 18S rDNA DGGE), the degradation of ^{14}C-labeled wheat straw or uniformly ring-labeled 2,4-dichlorophenoxyacetic acid, the development of the fungal biomass (ergosterol quantification) and the chemical composition of the soil organic matter (^{13}C CP/MAS NMR) were investigated. In half of the microcosms the soils were autoclaved and reinoculated with native soil, with intention to enhance the microbial growth.

Radiation induced shifts in the microbial community structure could be observed in all experiments. Some species were directly inhibited which could be seen by a loss of bands in the DGGE gels. Other species benefited from the radiation. The loss of competitors and thus a better nutrient supply are supposed to cause these effects. However, a radiation induced impact on microbial functions could only be seen in the 2,4-D mineralization experiment. The mineralization of the uniformly ^{14}C-ring-labeled herbicide 2,4-D was delayed for 4 days. Compared to the mineralization of wheat straw, only a limited amount of different species

participate in the degradation of the dichlorophenyl ring of the 2,4-D. It is suggested that these species were impacted by the radiation. Radiation induced impacts neither could be seen in the degradation of wheat straw, the chemical composition of soil organic matter (SOM) nor in the development of the fungal biomass. A redundancy of microbial functions is suggested to be responsible for that. In soils with high microbial diversity, certain functions are covered by different species, so that the disappearance of some species has no effect on the functions.

Effects caused by the sterilization and reinoculation with native soil prevailed in all experiments. Contaminations with ^{137}Cs or ^{90}Sr up to 50-fold that of the Chernobyl hotspots led to minor changes in soil microbial functions suggesting a strong resilience of natural soils towards radioactive contamination.

Kurzfassung

Auf lange Sicht gesehen sind die beiden Radionuklide ^{137}Cs und ^{90}Sr, welche in erheblichem Maße durch die Unfälle in Tschernobyl und Fukushima ausgestoßen wurden, die wichtigsten Verursacher für radioaktive Kontaminationen auf landwirtschaftlichen Flächen. Einmal im Boden angekommen können sie für mehrere Jahrzehnte an der Bodenoberfläche verbleiben. Aufgrund des hohen Gehalts an organischer Substanz, den höheren Temperaturen und dem Austausch von Assimilationsgasen kann hier in den oberen Bodenzentimetern die höchste Mikroorganismentätigkeit festgestellt werden. Aus diesem Grund sind die Mikroorganismen in den oberen Bodenschichten von Agrarböden besonders der Strahlung ausgesetzt. Es ist verwunderlich, dass es noch keine Untersuchungen gibt, in wie weit radioaktive Kontaminationen in realistischen Größenordnungen die mikrobiellen Populationen und ihre Funktionen in Böden beeinflussen.

In dieser Dissertation werden Inkubationsexperimente in Bodenmikrokosmen aufgeführt. Typische Ackerböden, eine Braunerde-Parabraunerde vom Standort Merzenhausen und eine schwach pseudovergleyte Parabraunerde vom Standort Kaldenkirchen-Hülst, wurden mit verschieden hohen Konzentrationen ^{137}Cs bzw. ^{90}Sr und teilweise mit radioaktiv markierten Substraten appliziert und unter kontrollierten Laborbedigungen inkubiert. Die niedrigsten radioaktiven Konzentrationen orientierten sich an der ^{137}Cs-Kontamination der Sperrzone um Tschernobyl. Die höchsten Radionuklidkonzentrationen lagen um den Faktor 50 über den maximal in Tschernobyl auftretenden ^{137}Cs Hotspots. In drei Experimenten wurden die Auswirkungen der ionisierenden Strahlung auf die Struktur der bakteriellen und pilzlichen Populationen (16S und 18S rDNA DGGE), der Abbau von ^{14}C-markiertem Weizenstroh bzw. 2,4-Dichlorophenoxyessigsäure (2,4-D), die Entwicklung der pilzlichen Biomasse (Quantifizierung des Ergosterolgehaltes) und die chemische Zusammensetzung der organischen Bodensubstanz untersucht (^{13}C CP/MAS NMR). Alle Mikrokosmenstudien wurden redundant durchgeführt. In einem Ansatz wurden native Ackerböden verwendet, in dem Anderen wurden die Böden autoklaviert und mit einem nativen Bodenaliquot angeimpft. Ziel war es, ein Wachstum der mikrobiellen Populationen zu erreichen und somit bessere Ergebnisse in den DGGE-Populationsanalysen ableiten zu können.

In allen Versuchen konnten strahleninduzierte Veränderungen der mikrobiellen Populationen festgestellt werden. Einige Spezies wurden direkt durch die Strahlung gehemmt, was an einem Verlust von Banden in den DGGE Gelen erkenntlich wurde. Andere Spezies wiederum

profitierten scheinbar von der Strahlung. Der Rückgang der strahlenempfindlicheren Arten erniedrigte den Konkurrenzdruck und erhöhte die Versorgung mit Nährstoffen für die resistenteren Arten. Dieser Effekt konnte sowohl bei den Bakterien, als auch bei den Pilzen festgestellt werden. Strahleneffekte auf mikrobielle Funktionen hingegen konnten nur im 2,4-D-Experiment festgestellt werden. Der mikrobielle Abbau des ^{14}C markierten 2,4-D-Phenylrings wurde um 4 Tage verzögert. Im Vergleich zu Weizenstroh sind am Abbau des 2,4-D-Phenylrings nur relativ wenige Spezies beteiligt, vermutlich wurden gerade diese von der Strahlung beeinträchtigt. Das Ausbleiben von Effekten in den anderen Experimenten wurde mit der Redundanz von mikrobiellen Funktionen erklärt. In Böden mit hoher mikrobieller Diversität werden bestimmte Funktionen von mehreren verschiedenen Arten abgedeckt, so dass der Wegfall einiger Arten keine Auswirkung auf die Funktionen hat.

In allen Versuchen hatte die Sterilisation und das anschließende Animpfen mit nativem Boden eine wesentlich größere Auswirkung auf die Mikroorganismen als die applizierten Radionuklide. Sogar Kontaminationen um den Faktor 50 höher als die maximal in Tschernobyl auftretenden ^{137}Cs-Hotspots führten nur zu sehr geringen Einflüssen, was eine hohe Widerstandsfähigkeit der mikrobiellen Populationen gegenüber radioaktiver Strahlung vermuten lässt.

Contents

List of figures

List of tables

Abbreviations

^{14}C	Carbon-14
^{137}Cs	Cesium-137
^{90}Sr	Strontium-90
AMF	Arbuscular mycorrhizal fungi
ECM	Ectomycorrhizal fungi
bp	Base pair
Bq	Becquerel
Ci	Curie
CO_2	Carbon dioxide
DGGE	Denaturing gradient gel electrophoresis
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
FES	Frayed edge sites
Gy	Gray
HAK	High-affinity-potassium transporters
HPLC	High pressure liquid chromatography
LSC	Liquid szintillation counter
Mzh	Merzenhausen
NaOH	Sodium hydroxide
NPP	Nuclear power plant
PCR	Polymerase chain reaction
R	Röntgen
rRNA	Ribosomal ribonucleic acid
TAE	Tris-acetate-ethylenediaminetetraacetic acid
TEMED	N,N,N′,N′-tetramethylethan-1,2-diamine
TN	Total nitrogen
TRK	A gene of Saccharomyces cerevisiae
UPGMA	Unweighted pair group method with arithmetic mean
VBNC	Viable but not culturable
WHC	Water-holding capacity

1 General introduction

Radionuclides are omnipresent in the environment. The main sources for naturally occurring radioactive isotopes are high energy cosmic rays and the earth's crust (Bilo et al., 1993; UNSCEAR, 2000). Important natural contributors to radioactive exposures are Radon-220, Radon-222 and Potassium-40 (UNSCEAR, 2000). However, due to nuclear weapon tests, several accidents at nuclear power plants and other nuclear facilities, artificial radionuclides were ejected into the environment. Up to the 1980s, the atmospheric nuclear weapon tests accounted for the most significant exposure of man-made radionuclides. Large amounts of radionuclides were ejected into the troposphere and even the stratosphere and dispersed with the wind. However, it is assumed that 50% of the volatile fission products, such as ^{90}Sr, ^{137}Cs and ^{131}I, deposited locally or regionally. The most active years for atmospheric bomb tests were 1954, 1958, 1961 and 1962. In 1962 the global deposition of ^{137}Cs was 4 kBq m^{-2} in the northern hemisphere. In the year 2000, 20 years after the last atmospheric bomb tests the global fallout decreased to 2 kBq m^{-2} (UNSCEAR, 2000). As most of the atmospheric bomb tests were carried out in the northern hemisphere; in the southern hemisphere the deposition with man-made radionuclides is considerably lower (UNSCEAR, 2000).

On April 26th 1986 the Chernobyl catastrophe happened. Concerning the dispersion of radionuclides, the situation in Chernobyl was unique, compared to other accidents at nuclear power facilities, even Fukushima. During a test-run intended to simulate the energy supply during a black-out, the reactor, which was built only three years earlier, exploded and the graphite, which was used as moderator, started to burn. Due to the heat of about 2000 °C, the ejected volatile radionuclides (e.g. cesium, tellurium, iodine and in low concentrations strontium) reached altitudes of about 1,500 to 10,000 m (UNSCEAR, 2000; Yablokov et al., 2009). During the subsequent two weeks, changing wind carried the radioactive plumes to Finland, Sweden and Western Europe, where the radionuclides fell out. About 40% of the European landscape were contaminated with at least 4 kBq m^{-2} ^{137}Cs (UNSCEAR, 1988). Maximum contamination in Western Europe reached 150 kBq m^{-2} in Austria (Bossew et al., 1996) and 184 kBq m^{-2} in central Sweden (Rosén et al., 1999). The maximum ^{137}Cs contamination in Germany was about 60 kBq m^{-2} (Konopleva et al., 2009). In Eastern Europe, in Belarus and the Ukraine the deposition rates rose to 20,000 kBq m^{-2} (Kashparov et al., 2003) or even 40,000 kBq m^{-2} at hotspots near the reactor (personal communication Dr.

Valery Kashparov, 2009, Ukrainian Institute of Agricultural Radiology). The Chernobyl accident was the first to be considered with level 7 on the International Nuclear and Radiological Event Scale **(**INES). The second accident that reached the maximum level at the INES was the core meltdown of several reactors in Fukushima, Japan in 2011.

Due to bomb-testing and accidents at nuclear power-plants and facilities, different radionuclides were deposited on terrestrial ecosystems. The bomb-fallout comprised large amounts of ^{90}Sr and ^{134}Cs (Dumat and Staunton, 1999; Scheffer et al., 2002), the Chernobyl fall-out consisted of fission and activation products such as ^{95}Zr, ^{95}Nb, ^{106}Ru, ^{137}Cs, ^{134}Cs, ^{90}Sr, isotopes of iodine and ^{144}Ce (UNSCEAR, 1996; Borovoi and Gagarinskii, 2001).

The amount of the total emissions from reactor 4 in Chernobyl is not exactly known. Borovoi and Gagarinskii (2001) assume that 90 MCi ($3.3*10^{18}$ Bq) of radionuclides with half-lives larger than 20 h have been released during the 10 days of explosion and burning. That is about 5% of the total radionuclide inventory originally existing in the reactor. The emissions can be divided into three different forms, condensed particles, volatile gases and particles bound to a fuel particle matrix, with all three influencing the dispersion in the environment (UNSCEAR, 2000). The volatile fission products such as alkali metals (cesium), iodine and tellurium were ejected gaseous and condensed at light aerosols. From the original ^{137}Cs inventory approximately 33 ± 10% has been released and was transported with warm airstreams across Europe (Borovoi and Gagarinskii, 2001). The same applies to the volatile gases. In case of the fuel particle matrix the size plays an important role for its dispersion. Hot particles consist of parts of the radioactive fuel (uranium and plutonium) melt together with other radionuclides such as ^{90}Sr. The hot particles distributed over Europe had an average size of 15 µm, with the larger particles being deposited closer to the reactor. For distances below 30 km from the reactor core, contaminations with ^{90}Sr prevail, whereas beyond that zone the contamination is mainly caused by ^{137}Cs (Kashparov et al., 2004; Yablokov et al., 2009).

The effects of ionizing radiation on human health have been the content of numerous studies. A major aspect within these studies was the transfer of airborne radionuclides from soil to plants and finally into the human food chain (Bilo et al., 1993).

Although several scientists investigated the effects of ionizing radiation on microorganisms already before the Chernobyl accident (Davis et al., 1956; Monib et al., 1971; Franz and Woodwell, 1973; Gochenaur and Woodwell, 1974), the participation of micro-organisms in transport and fate of radionuclides in soil was disregarded for a long time. Thus, in numerous

experiments soil samples have not been considered as habitat for bacteria and fungi. The samples often were treated roughly, were sterilized, heated and ground. During these treatments the majority of organisms may die or fall into a viable-but-not-culturable (VBNC) state (Sanchez et al., 2000). However, the fate of radionuclides may depend on soil microorganisms, and vice versa, the radionuclides may affect the microorganisms.

2 Fate and effects of radionuclides in soils, state of knowledge

2.1 Microbial functions in agricultural soils

One of the basic microbial functions in soil is the mineralization of organic substances. Bacteria and fungi degrade crop residues and manure and release nutrients such as nitrogen, phosphorus, carbon dioxide and mineral elements. In addition to herbal and animal organic matter, most of the agricultural areas are treated with organic xenobiotics. Each year between 33.000 and 44.000 t of pesticides are sold in Germany (Bundesministerium für Ernährung, 2010). Here the microorganisms play an important role in groundwater protection as they degrade the pesticides before they reach the aquifer. Once having reached the aquifer, the mineralization of the pesticides is almost stopped due to anaerobe conditions (Stroh, 2006). In addition to mineralization of organic matter another important microbial function is the formation of soil organic matter. Particularly the more recalcitrant compounds such as lignin and hemicelluloses are transferred into humic substances (Scheffer et al., 2002). Humic substances have important characteristics in soils; they enhance the cation exchange capacity, they serve as supporters for nutrients, especially carbon and they are able to bind heavy metals. Furthermore they improve soil structure, water-holding capacity and they darken the soil surface color, thus they are positively affecting the heat balance of soils (Scheffer et al., 2002). Soil organic matter and indirectly soil microbial activity are decisive factors ensuring the fertility of agricultural soils.

2.2 The radionuclides ^{137}Cs and ^{90}Sr, physical characteristics

Cesium an alkali metals belongs to the first group in the periodic table. It is quite reactive and does not exist in the elemental form in nature. Cesium exhibits 39 isotopes of which only ^{133}Cs occurs naturally. Most of its isotopes have very short half-lives, whilst ^{137}Cs has a half-life of 30.17 years (Unterweger, 2002). ^{137}Cs emits β- and γ-rays with 95% of the beta-emission consisting of energy of 0.511 MeV and the remaining part consisting of 1.173 MeV. The gamma-radiation averages to 85% of 0.662 MeV (Parekh et al., 2008). ^{137}Cs is generated in the explosion of atomic weapons and in nuclear power plants as a fission product (Volkmer, 2007). Radioactive cesium in soil is mainly fixed due to adsorption to clay minerals, particularly illites. Presumably, the adsorption process is specific, as the clay

minerals contain a small part of specific bounding sites, so called frayed edge sites (Smolders et al., 1997). Cesium belongs to the same group in the periodic table as potassium and has a similar atomic radius as K-atoms. The strength of fixation is caused by interlayer collapse of the clay minerals. Cesium features lower hydration energy than potassium and thus it is easier to strip down the hydration sheath. When water leaves the intermediate layer, the clay mineral collapses and the Cs-atom is strongly fixed (Dumat and Staunton, 1999). Under normal conditions occurring in soils, fixed cesium is no longer available for microorganisms (Steiner et al., 2002). However, in soils with large organic matter content and low content of clay, cesium is less strongly adsorbed. The migration rate is analogous to mineral soils, but the bioavailability is much higher (Dumat and Staunton, 1999). Kruyts and Delvaux (2002) assume that the abundance of frayed edge sites (FES) directly regulates the mobility of ^{137}Cs and that the accumulation of organic matter in top soil acts as a diluting effect on FES bearing material. In contrast to cesium, strontium behaves differently in soils. Strontium is an alkaline earth metal and belongs, just like calcium, to the second group in the periodic table. It is very reactive and occurs in nature only as a chemical compound. In the long term, for ecological issues, the most important isotopes of strontium are ^{90}Sr and its decay product ^{90}Y, which is in a steady equilibrium with ^{90}Sr. ^{90}Y exhibits a half-life of approximately 60 hours only, but its beta-decay increases the beta emission of ^{90}Sr. The decay energy of ^{90}Sr and ^{90}Y are 0.546 MeV and 2.282 MeV, respectively (Keller, 1993). ^{90}Sr (and ^{90}Y) is one of the most high-energy beta-ray emitting isotopes with a long half-life of 28.78 years. ^{90}Sr is, as well as ^{137}Cs a nuclear fission product. Since it is similar to calcium, strontium plays an important role for ecological and biological considerations (UNSCEAR, 1996). In contrast to cesium, strontium is very mobile in mineral soils. It is not being adsorbed to specific binding sites on clay minerals and thus can be easily exchanged by ion exchange mechanisms. ^{90}Sr normally shows faster downward migration in mineral soils than cesium does (Ivanov et al., 1997; Gastberger et al., 2000). The mobility of strontium in soil mainly depends on the amount and quality of organic matter, the content of exchangeable calcium and the pH value (Coughtrey and Thorne, 1983; Wauters et al., 1996). High amounts of exchangeable calcium and high pH decrease the downward migration of strontium (Wiklander, 1964). In organic soils, large quantities of strontium are bound to fulvic acids. Increasing amounts of organic matter reduces the plant uptake of strontium (van Bergeijk et al., 1992). In soils dominated by mineral components, strontium is mainly bound to weak regular exchanging sites, which are not specific (Mueller-Lemans and Van Dorp, 1996; Jones et al., 2004).

2.3 The role of microorganisms for retention and transport of ^{137}Cs and ^{90}Sr

Within soils rich in clay minerals, cesium is fixed rapidly and is no longer bioavailable. Remarkably, in forest or fen soils, with a low clay content at the surface layer (organic matter > 95%), the migration rates seem to be similar to mineral soils (Dumat and Staunton, 1999; Kruyts and Delvaux, 2002; Steiner et al., 2002) with most of the deposited ^{137}Cs being found in the top soil layer (Auerbach, 1986; Drissner et al., 1998; Rosén et al., 1999; Konopleva et al., 2009). Typical distribution patterns of radiocesium in a forest and a grass land soil can be seen in Figure 2.1. Organic soils are not able to bind cesium or strontium physico-chemically (Lieser and Steinkopff, 1989), which leads to the conclusion that microorganisms (Brueckmann and Wolters, 1994; Parekh et al., 2008) and particularly fungi (Delvaux et al., 1996; Rafferty et al., 1997; Fukuyama and Takenaka, 2004) play an important role in the immobilization and upward transport of cesium isotopes in organic forest soils. In undisturbed forest soils up to 75% of the living biomass consists of fungal microorganisms (Kohlmeier et al., 2005). In central European forests most of the plants live in association with fungal mycorrhiza. The host plants supply the fungi with carbohydrates, spending up to 20% of their photosynthesis products, while the fungi enhance the nutrient supply for the plants (Smith and Read, 2008). The surface area of fungal mycelium is usually much larger than the root system of the plants. The very thin hyphae can reach nutrient pools, which are not available for roots and the mycelium penetrates the soil with much lower amount of energy (Steiner et al., 2002). This enhances the supply with nitrogen, phosphorus and water in nutrient poor habitats. So, Smith and Read (2008) conclude, that the mycorrhizal mycelium is the main organ for taking up nutrients of terrestrial plants. However, in relation to the enhanced transport of nutrients, the transport of other elements such as toxic substances and radionuclides also has to be considered. The role of fungi in transport and accumulation of radionuclides has been subject of numerous investigations. It has been suggested, that fungi store the majority of radio-cesium in forest soils (Dighton et al., 1991; Brueckmann and Wolters, 1994; Guillitte et al., 1994; Vinichuk and Johanson, 2003).

Excursus to fungal Mycorrhiza:

In central European forests mainly two mycorrhizal types interact with trees and higher plants; the arbuscular mycorrhizal fungi (AMF) and the ectomycorrhizal fungi (ECM) (Smith and Read, 2008). Ectomycorrhizal fungi are characterized by a fungal sheath, which encloses the plant root, inward growing hyphae between the epidermal and cortical cells (named Hartig net) and an extraradical mycelium which connects the roots with the soil and the sporocarps. Almost all plants living in symbiosis with ECM are perennial plants (Smith and Read, 2008). The ectomycorrhiza appears in upper organic horizon or in the interface between mineral and organic horizon (Smith and Read, 2008). The accumulation and the transport of radionuclides in ectomycorrhizal fungi is much better observed than in AM fungi. In fungal sporocarps the ^{137}Cs activity was found to be 10 to 270 times higher compared to plants growing at the same location (Bakken and Olsen, 1990; Yoshida and Muramatsu, 1998). However, the role of fungi concerning the transport of cesium into the mycorrhizal partners is not unequivocally determined. In some studies enhanced transport was found (Drissner et al., 1998; Strandberg and Johansson, 1998; Ladeyn et al., 2008), in others the fungi inhibited the cesium transport into the host plants (Clint and Dighton, 1992; Riesen and Brunner, 1996). Latter authors assume that ECM fungi absorb the radiocesium and reduce its availability for plants. However, it should be noted that ECM seem to be the major contributor for radiocesium immobilization in organic forest soils (Dupré de Boulois et al., 2008).

In contrast to ECM, the arbuscular mycorrhizal fungi penetrate the root cell and form an intracellular hyphae, coils and arbuscules. These arbuscules act as main site for exchanging nutrients between the plant roots and the fungi (Garg and Chandel, 2010). Unlike the ECM, the AM fungi usually occur in habitats dominated by herbaceous plants (Dupré de Boulois et al., 2008). Some studies observed the role of AM fungi for potassium and radiocesium transport and accumulation. However, the findings cannot be summarized unambiguously. Rosén et al., (2005) observed an enhanced uptake of ^{137}Cs by leek cultivated in the greenhouse, while no uptake into ryegrass was observed in the same experiment. Berreck and Haselwandter (2001) noted a decreased cesium uptake in bentgrass (Agrostis tenuis). They assumed that the fungi sequestrate the Cs in the extraradical hyphae and do not transfer it into the plant root. Joner et al. (2004) did not observe any involvement of AMF in transporting ^{137}Cs into plants. Presumably

chemical, physical and biological factors regulate the uptake of nutrients and cesium. Dupré de Boulois et al. (2008) concluded in their review that the uptake of cesium by AMF could be "strongly impaired by the sorption capacity of clay, the bio-availability of K in the soil solution and /or plant K nutritional status." Indeed, the way how and when the fungi take up the nutrients, particularly K^+ and Cs^+ is not clearly identified. At least two K transporters for fungi are known, the high-affinity-potassium transporters (HAK) and the TRK-transporters (TRK is a gene of Saccharomyces cerevisiae). The former ones seem to exhibit a low selectivity between K- and Cs-ions and are responsible for uptake at lower K concentrations. The TRK transporters are more selective against Cs^+ and mainly work at higher K^+ concentrations in the substrate (Zhu and Smolders, 2000; Dupré de Boulois et al., 2008).

The findings of Berreck and Haselwandter (2001) and Declerck (2003) indicate that a part of the Cs taken up by the fungi presumably will be accumulated in the fungal mycelium. But the findings are not clear; as the effects may also be rooted in the experimental setup. However, unlike the ECM fungi, the AMF do not develop sporocarps or fruitbodies which seem to accumulate more radiocesium compared to the remaining mycelium. In case of the AMF, the intraradical structures seem to act as local storage compartments for radiocesium (Dupré De Boulois et al., 2005).

In contrast to fungi, information about bacterial transport of radionuclides is sparse. Except for flagellates, bacteria are not able to transport radionuclides actively across wider ranges (Kohlmeier et al., 2005). However, due to ingestion or biotransformation, bacteria can influence the transport mechanisms. Bacteria can act as pseudo-colloids and they are able to form aggregates with other colloidal material. Thus the radionuclides incorporated by bacteria could be transported in soil with water flow or colloidal transport mechanisms (Gadd, 1996).

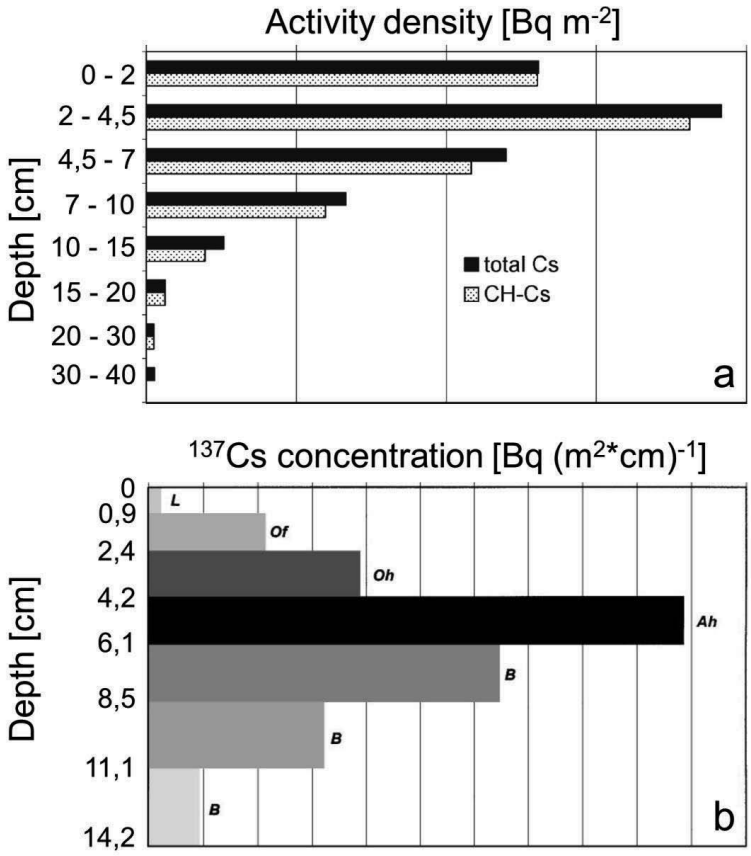


Figure 2.1: Typical depth distribution of ^{137}Cs in soil of a: an undisturbed grassland in 1990 (Schimmack and Schultz, 2006) and of b: a spruce forest soil in 1994 (Drissner et al., 1998), figures modified. Ch-Cs: Chernobyl derived ^{137}Cs.

2.4 Radionuclides in forest ecosystems and agricultural soils

Most of the knowledge concerning the transport and behaviour of radionuclides is built on studies conducted in forest ecosystems. However, there are large disparities between forests and agricultural ecosystems. The transfer of radionuclides in forest soils is much higher than in cultivated crop soils. In forests radionuclides can be stored and recycled with high efficiency, which results in long residence times (Calmon et al., 2009). Due to the low content

of clay minerals in upper soil layers and high organic matter content, the radionuclides circulate in a bioavailable form. The lack of short-term cultivation and large deposition of organic matter leads to a litter accumulation in varying thickness (Guillitte et al., 1994) and a heterogeneous structure of plants with various root depths. This results in a heterogeneous distribution of radionuclides in forest soils (Konopleva et al., 2009).

The grade of decomposition of the litter is a significant driver for the downward transport of cesium. Litter decomposition and humus production depends on the compounds and the nitrogen content. The decomposition rate for coniferous litter is low (little nitrogen, lots of lignin and antibacterial ingredients) and thus coniferous forests form a thick humus layer which can accumulate large amounts of bioavailable radionuclides. This layer is the main source for plant root uptake of cesium (Konopleva et al., 2009).

Compared to forest soil, arable soils are different as they are cultivated regularly. Plowing for example leads to a homogenous distribution of radionuclides, and harvesting avoids the accumulation of a pronounced humus layer. Crop soils are often rich in clay near the surface and thus most of the radiocesium is rapidly bound to the selective site of the mica and is no longer available for plants and microorganisms (Konopleva et al., 2009). However, the downward migration of radionuclides, particularly radiocesium is low or even negligible in forest and agricultural soils. Only in the first days after deposition appreciable leaching losses can be expected (Rafferty et al., 2000).

2.5 Effects of radionuclides on microorganisms

Radionuclides affect microorganisms in several ways. The effects depend on the dose, the durability, the physicochemical factors, the role in the metabolism and the deposition form of the radionuclides (Haas, 2001). In arable soils, additional soil treatments such as plowing, liming and fertilizing can influence the radionuclide availability and thus the effects of radionuclides. For radionuclides, it is necessary to distinguish between toxic effects as well as direct and indirect damage caused by ionizing radiation. In contrast to other radionuclides and heavy metals the chemical toxicity of ^{137}Cs and ^{90}Sr can be neglected (Gadd, 1996). Direct damage in cells is caused by high-energy particles or photons, which break structural bonds by collision with molecules. This may lead to single-strain and double-strain-breaks of DNA and destruction of proteins (Daly, 2009). Indirect damages result from high energy particles

which move through extracellular or intercellular water (McNamara et al., 2003). Radiolysis generates free radicals, which leads to the formation of hydrogen peroxide (H_2O_2). Hydrogen peroxide is highly reactive and can cause lesions and damages within the DNA and other molecular structures in cells (Nicastro et al., 2002; Daly, 2009). Precondition for damage caused by free radicals is the ionization of water close enough to the DNA molecule (Nicastro et al., 2002). Korystov (1992) compared the effects of ionizing radiation to dry and wet cells. He disclosed that water radiolysis products cause 85-90% of damages caused by ionizing radiation. Besides the water content in the microbial cells, the soil moisture determines the radio-sensitivity of the microorganisms. In their review, McNamara et al. (2003) conclude that dry soils need higher doses of gamma irradiation for complete sterilization than wet soils. However, in this case the physiological state of the microorganisms has to be considered as well; in dry soils the microbial population may form spores or fall into the viable-but-not-culturable state (VBNC). In this state the metabolism is reduced and the mechanisms for DNA repair are working more effectively (Pitonzo et al., 1999b, a). Therefore, bacteria are more tolerant against extreme environment conditions such as heat, drought and ionizing radiation.

The effects of ionizing radiation and other environmental impacts can be derived from alterations in the microbial community structure as well as the change in microbial functions.

2.5.1 Radiation effects on microbial communities

Relative little attention has been awarded to the effects of low-dose radioactive contaminations on microbial communities. Most of the available studies deal with the irradiation of samples or soils with high dose rates. Here the radiation is often used for sterilization of soils prior to experiments (McNamara et al., 2003) or for the sterilization of food and seeds. The employed dose rates usually were in the range of several kGy (Gy = Gray; absorbed radiation dose), which is away from any realistic dose rates found in the environment due to accidents. As a consequence of these sterilization-experiments, the lethal doses for several isolated strains are well known. Gamma-irradiation doses below 10 kGy are called sub-sterilizing doses and have a lethal effect to most of the actinomycetes, fungi and invertebrates. The majority of bacteria is eliminated at 20 kGy and doses above 70 kGy kill radio resistant bacteria (McNamara et al., 2003). However, the knowledge of the various tolerances against radiation is mostly related to isolated microbial strains in pure culture under

optimal cultivation conditions. Under realistic conditions the soils exhibit a complex and diverse micro-flora with predator-prey interactions, association and competition within and between the species (Fuma et al., 2003) as well as manifold biotic and abiotic impacts. One gram of soil exhibits up to one billion bacteria and 5000 different species (Amann et al., 1995). Most of them are unknown and only few of them (0.1% - 10%) can be cultivated (Scheffer et al., 2002; Schlegel et al., 2007). Environmental changes and impacts on several species may affect the whole community structure. Already in 1956 Davis (Davis et al., 1956) concluded from his radiation experiments with a microbial population in a tree hole habitat that it is sufficient to destroy one segment of a population to reach an unbalance in the whole microbial community. To examine the interactions in irradiated soils Fuma et al. (1998) exposed an artificially assembled microbial community to various doses of gamma radiation. The community consisted of three compartments, one algal species (*Euglena gracilis*) as producer, one protozoan species (*Tetrahymena thermofila*) as consumer and one bacterial species (*Escherichia coli*) as decomposer. Increasing gamma-irradiation first led to a temporal inhibition and then to total inhibition of the activity of *E. coli.* Subsequently the amount of the algae decreased although the irradiation dose was below its effective dose. The authors assume that the reduction of *E. coli* caused by the ionizing radiation impacts the other organisms due to their dependence on bacterium metabolites. Although the microcosm experiment of Fuma is just a simple model, it demonstrates the interactions between various organisms in soil.

Species in the communities exhibit various tolerances and resistances against ionizing radiation and thus responses differently to stresses and strains. A direct relationship between microbial diversity and microbial functions is not always visible (Griffiths et al., 2000; Griffiths et al., 2004). In soils with high microbial diversity, the effects of stress are less pronounced, particularly for general functions (e.g. decomposition rate of plant residues, growth on added nutrients and thymidine incorporation). This can be explained by a functional overlap due to different species with similar functions. In contrast, specific functions (e.g. nitrification, denitrification, methane oxidation) which depend on just a few species are more sensitive to stress (Chapin, 1997; Griffiths et al., 2000). One should consider that the chloroform fumigation method Griffiths et al. used is a very harsh method and it is assumed that nearly all species are influenced similarly. In contrast, the radiation effects seem to be more selective, as different species response distinctly to ionizing radiation and exhibit

different radio resistance as well as radio tolerance. It is necessary to differ between stresses directly caused by ionizing radiation and stresses indirectly evoked by radiation injury of other organisms. An example for indirectly caused stresses can be seen in the study of Jones et al. (2004). They irradiated a *Holcus lanatus* dominated grassland soil in the range from 5 to 160 Gy. At lower doses up to 80 Gy the amount of gram-positive bacteria increased, while gram-negative bacteria, such as *Pseudomonas,* were reduced. Mycorrhizal fungi disappeared completely at these doses. Jones et al. (2004) explained the increase of gram-positive bacteria with additional releases of root exudates due to damages of the *Holcus lanatus*. The more resistant bacteria, in this case gram-positive ones, could survive and benefit from lower competition and increased nutrient supply due to cell lysis products emitted by the killed gram-negatives and the fungi. In contrast, in an experiment conducted by McNamara et al. (2007) the gram-negative bacteria showed higher radio-tolerance. Using the denaturing gradient gel electrophoresis (DGGE) they compared the microbial community structure between soil samples irradiated with different doses. At doses in the range of 10 kGy new bands occurred in the gel pattern, which previously did not appear at lower doses. The formation of these discrete new bands was also explained with indirect effects on competition and nutrient support. The increase of the new bands correlated to the decrease of fungal community, so they concluded that niche competition was an important driver in their experiments.

The duration of radioactive contaminations in the upper soil layer is limited by the physical half-life and the loss of radionuclides, e.g. due to migration into deeper soil layers (Alexakhin et al., 2007). In case of ^{137}Cs and ^{90}Sr the half-lives are relatively long and the mobility is low, thus the microorganisms in the upper soil layer may be exposed to ionizing radiation exceedingly and for long periods. Gochenaur and Woodwell (1974) observed the changes in a soil micro fungi community in a forest soil, which was irradiated with gamma-rays for seven years. The distributional pattern of the fungal community was correlated with the radiation dose. Near the γ-source, where the highest doses reached 1800 R day^{-1} (~ 18 Gy d^{-1}), 2 cm below the soil surface only slow growing sterile fungi were found[1]. The population was characterized by intensely melanized fungi, which had dark colored hyphae. The morphology

[1] For gamma-radiation 1 R is about 0.01 Gy (Volkmer, M., 2007. Basiswissen Kernenergie. Informationskreis Kernenergie, Berlin.)

was simple and they produced just a rudimentary mycelium. The fungi had simple nutritional requirements; they could use nitrate and glucose as sole nitrogen and carbon source respectively. With increasing distance to the radiation source the hyphae became more hyaline and the structure and morphology became more complex.

Bacteria are able to produce 40 generations per year (Romanovskaya et al., 1998). As a result of the high reproduction rate, they have the capability to adapt fast to changing environmental conditions via selection and mutation. Eriksen and Emborg (1978) cultivated different radio resistant bacteria from soils, which were irradiated with a gamma-source for 18 month. Repeated irradiation after few weeks caused a shift in microbial community. Bacteria, which were irradiated before with high doses, showed a better growth than the lower irradiated reference group. The authors did not examine the mechanisms, however it is assumed that the surviving bacteria adapted to the radiation due to mutation. Remarkably, all isolated bacteria were gram-positive and intensively red-pigmented. Additional literature also suggests, that dark pigmented organisms, fungi as well as bacteria, seem to be more radio resistant than non-pigmented ones (Rokitko et al., 2003; Dadachova et al., 2007). A further example for good adaptation of gram-positive bacteria to radioactive environment can be seen in the studies of Fredrickson et al. (2004). They observed the microbial community of an intensely contaminated soil at the Hanford Site, a nuclear production complex in the US state Washington. Forty-two years after leakage of a tank filled with a mixture of various radioactive chemicals they isolated different bacteria and fungi from highly contaminated soils (e.g. > 0.74 MBq $^{137}Cs\ g^{-1}$). Most of the isolated species were inactive or in a dormant state but some species could be cultivated. The majority of these bacteria was gram-positive and had a high content of guanine and cytosine, which is typical for organisms living in vadose zones. The finding of cultivable bacteria demonstrates that some phyla are very effective in surviving in extremely contaminated areas.

2.5.2 The impact of ionizing radiation on microbial functions

Carbon decomposition and soil respiration are good indicators for microbial activity in soil (McNamara et al., 2003). Different species prefer different carbon sources. This behavior is accelerated in stress situations such as irradiation. Pitonzo et al. (1999a) examined the carbon consumption of various micro-organisms irradiated with different doses of up to 9.34 kGy. With increasing irradiation, the metabolic activity decreases and microorganisms started to

use the simpler carbons such as simple sugars and amino acids. The non-irradiated species used carbon sources from all major groups represented on the BIOLOG® microtiter-plates. The authors did not investigate the mechanisms, however, possible explanations are, that the limited energy reserves were used for DNA repair rather than for enzyme production, or that the enzyme production is affected by DNA damages caused by the radiation (Pitonzo et al., 1999a). The reduction in metabolic activity led to reduced decomposition of complex carbon sources, thus potentially affecting the degradation of soil pollutants such as herbicides and pesticides. *Pseudomonas* species for example, whose cell number was reduced in an experiment conducted by Jones et al. (2004), are important, gram-negative degraders of Atrazine (Mandelbaum et al., 1995).

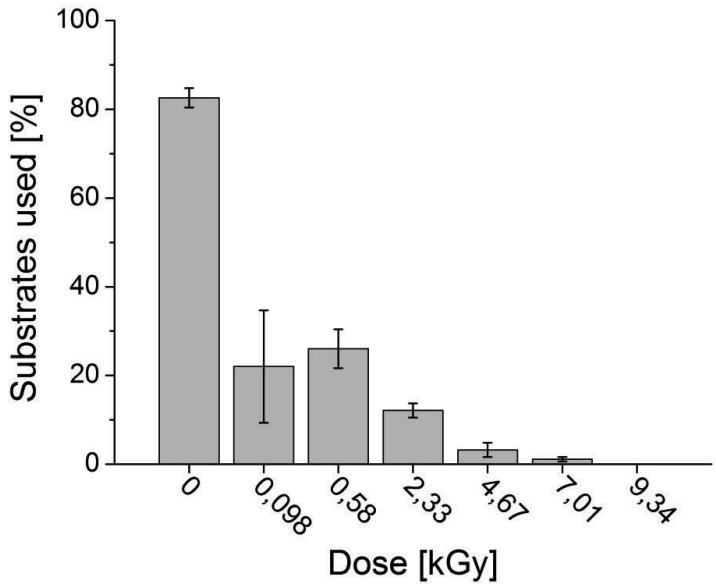


Figure 2.2: Amount of carbon substrates used throughout gamma irradiation by microorganisms resuscitated from the VBNC state. Determined by BIOLOG (*n = 180*). Generated after Pitonzo et al. (1999a).

Besides the carbon turnover, other functions such as ammonification and nitrification are also affected by ionizing radiation. In their review article, McNamara et al. (2003) describe an increase of ammonium and ammonia accumulation and a decrease of nitrification in irradiated

soils. In all reviewed studies, the soil was treated with gamma rays in a range from 5 to 50 kGy, apart from one, which was irradiation with 200 Gy. Directly after irradiation, the ammonium content increased, whilst the nitrate content decreased. The authors explained these findings with the formation of peroxides and the reduction of nitrifying bacteria (Thompson, 1990; Lensi et al., 1991). However, the effects on ammonifying and nitrifying microorganisms are not consistent across the different experiments described in scientific literature. In a long term experiment, carried out by Poinsot-Balaguer et al. (1991), the nitrate concentration in soil increased due to ionizing radiation. The authors assumed that, particularly at relatively low doses, the indirect radiation effects prevail, which themselves affect the microbial activity (compare to Jones et al., 2004) and thus the nitrate production.

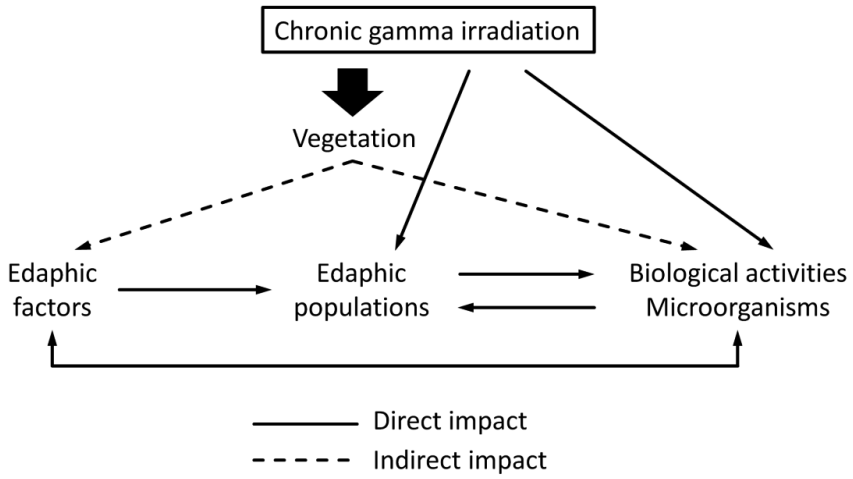


Figure 2.3: The impact of chronic gamma irradiation after 16 years' exposure of soil systems at Cadarache. The figure demonstrates the strong influence of radiation to the vegetation and subsequent to the soil micro-flora. Modified according to Poinsot-Balaguer et al. (1991).

2.6 Thesis outline

Microorganisms in soil play an important role for the nutrient cycle, decomposition of nitrogen and carbon as well as soil pollutants. They are able to mobilize or immobilize radionuclides (Simonoff et al., 2007) and enhance the radionuclide-retention to soil organic matter (Sanchez et al., 2000). Due to higher contents of organic matter, warmer temperatures and a gas exchange with the atmosphere, the majority of these processes is working in the upper 10 cm of soil (Jones et al., 2004). Hence, the upper soil layer is most susceptible for radioactive contaminations. After accidents most contaminants eventually deposit on the soil or the plant surface and infiltrate the soil. According to literature, the migration of ^{90}Sr, and especially ^{137}Cs into deeper soil layers is rather slow (Zhu and Shaw, 2000; Kruyts and Delvaux, 2002; Konopleva et al., 2009) and both radionuclides usually remain in the upper soil layer for several decades (Drissner et al., 1998; Rafferty et al., 2000). Thus, alternating effects between microorganisms and radionuclides are probable.

As a result of research conducted since the 1940s and acute research after the Chernobyl accident, it is well known how to reduce the uptake of radionuclides into plants and into the human food chain (Zhu and Shaw, 2000). However, no data are available on, how ^{137}Cs and ^{90}Sr in realistic concentrations, e.g. around the damaged reactors, impacts soil microorganisms. The applied doses used in scientific experiments were usually much higher and amounted up to several kGy (Pitonzo et al., 1999b; McNamara et al., 2007). Therefore, it is important to enlarge the knowledge base in radioecology in terms of realistic radiation doses, as to respond correctly to accidents and terroristic acts. Moreover such new findings would also help to assess the risks of contamination accurately.

In a series of experiments the effects of radioactive contaminations on the microbial community in soil and some of its functions were observed. This thesis is mainly based on peer-reviewed publications. The chapters 3 - 5 deal with experiments, which were conducted in the controlled area of the Agrosphere Institute (IBG-3), Forschungszentrum Jülich GmbH. All experiments were conducted in soil microcosms which were artificially contaminated with various concentrations of ^{137}Cs and ^{90}Sr. For the investigation of the mineralization activity of the microorganisms ^{14}C-labeled substrates were applied. The evolving $^{14}CO_2$ was absorbed in NaOH-traps and measured in the Liquid Scintillation Counter (LSC).

In chapter 3 the impact of ^{137}Cs or ^{90}Sr on the mineralization of ^{14}C-labeled wheat-straw is discussed. The microcosms were incubated for 70 days at 20 °C. Changes in the fungal as

well as bacterial community structure were examined with help of the denaturing gradient gel electrophoresis (DGGE). To explain the effects of sterilization and reinoculation on the bacterial community structure and the degradation of wheat straw, a supplementary experiment was conducted. The results were not published, but detailed information is provided in chapter 3.7.

In chapter 4 the effect of ^{137}Cs and ^{90}Sr on the development of fungal biomass and chemical composition of soil organic matter is investigated by quantifying the ergosterol content and solid state NMR technique (^{13}C CP/ MAS NMR), respectively. Furthermore, the bacterial and fungal communities were compared with help of the DGGE.

In chapter 5 the investigations focus on the influences of the applied radionuclides on the mineralization of the ^{14}C-labeled herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). The microcosms were incubated for 30 days at 20 °C. The 2,4-D mineralization was investigated by determining the released $^{14}CO_2$. Shifts in the microbial community structure were observed with the DGGE.

3 Effects of low level radioactive contamination and sterilization on the degradation of radiolabeled wheat straw[2]

3.1 Objectives

The objectives of the experiment were to observe the effects of soil contaminations with ^{137}Cs and ^{90}Sr on the mineralization of ^{14}C-labeled wheat straw and the fungal as well as bacterial population. Wheat straw is a crop residue and is used as fertilizer. It is known that a large diversity of microorganisms participates in the degradation in several steps. For determining the mineralization activity of the microorganisms the emitted $^{14}CO_2$ was absorbed in NaOH and measured in the LSC. The alterations in the microbial community structure were determined with the 16S and 18S rDNA DGGE technique.

3.2 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 (Pitonzo et al., 1999b; McNamara et al., 2007). Due to these experiments, which usually involved sterilization of the soils, the

[2] Adapted from: Niedrée, B., Vereecken, H., Burauel, P., 2012. Effects of low-level radioactive soil contamination and sterilization on the degradation of radiolabeled wheat straw. Journal of Environmental Radioactivity 109, 29-35.

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. Gochenauer 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 km² 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 remediation of such huge areas is extremely expensive and the natural migration of several radionuclides is low (Thiry and Myttenaere, 1993; Zhu and Shaw, 2000; Konopleva et al., 2009), it is important to know how radionuclide contamination affects the soil microflora, 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: 0.1m). 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 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.

3.3 Material and Methods

3.3.1 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 Rur River system, covered by Pleistocene and Holocene eolian sediments. The used soil from the plowing 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)).

3.3.2 Soil sampling and sample preparation

Soil samples were taken from the plow 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 (≤ 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 hours. The sterilized soil was dried at 105 °C for 24 hours. 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 (McNamara et al., 2003; Berns et al., 2008). 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 x 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.

3.3.3 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 hours. 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 ultracentrifugal mill at 15,000 rpm (Retsch, Germany). The soil-straw mixture was thoroughly mixed in an end-over-end shaker for 45 min. 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.

3.3.4 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 8ml 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 over the whole time of incubation. Water loss was controlled continuously by weighing, and water was added when necessary. The microcosms were incubated at 20 ± 1°C for 70 days.

3.3.5 DGGE

DNA was extracted with the extraction kit "FastDNA Spin Kit for Soil, BIO101" from Qbiogene, Carlsbad, USA. The mixture for amplification of 16S rDNA sequence contained 5 µl reaction buffer (750 mM Tris-HCl, pH 8.8 at 25°C, 200 mM $(NH_4)_2SO_4$, 0.1% (v/v) Tween, 15 mM $MgCl_2$), 2.5 µl DMSO, 0.4 µl hot-start polymerase and 2 µl of each primer. Chemicals for amplification were purchased from Thermo Scientific (Waltham, Massachusetts); DMSO from Carl Roth (Karlsruhe, Germany). Primers were purchased from Eurofins MWG Operon (Ebersberg, Germany). The vials were filled up with sterile MilliQ

water to a total volume of 50 µl. As specific primers for eubacterial 16S rRNA genes 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. After activation of the hot-start polymerase for 10 min at 95°C, the polymerase chain reaction (PCR) ran in 35 cycles, consisting of 1 min at 94°C, 1 min at 54°C and 1 min at 72°C, followed by a single period of 10 min at 72°C (Thermocycler iCycler IQ by Bio Rad Laboratories, USA).

For the 18S rDNA amplification, the same DNA template was used as for the 16S rDNA amplification. The mixture for amplification contained of 1 µl DNA template, 5 µl reaction buffer (750 mM Tris-HCl, pH 8.8 at 25°C, 200 mM $(NH_4)_2SO_4$, 0.1% (v/v) Tween, 15 mM $MgCl_2$), 3 µl 25 mM $MgCl_2$, 1.5 µl DMSO, 0.4 µl hot-start polymerase and 1 µl of each primer. The vials were filled up with sterile MilliQ water to a total volume of 50 µl. Primers specific for fungal RNA were chosen. Primer NS1 (5'-GTA GTC ATA TGC TTG TCT C -3') and GCFung (CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC CAT TCC CCG TTA CCC GTT G-3') are known to amplify fragments with 350 bp length (Muyzer et al., 1993; May et al., 2001). Primer GCFung included a 40-base GC clamp for enhancing the melting stability of the PCR products in the DGGE (Ferris et al., 1997).

Denaturing gradient gel electrophoresis was performed with a Dcode Universal Mutation Detection System (BioRad, USA). In the case of 16S rDNA, a 6% polyacrylamide gel was used (6% [w/v] acrylamide, 0.1% [v/v] TEMED [N,N,N′,N′-Tetramethylethan-1,2-diamin], 0.1% [w/v] ammonium persulfate). The electrophoresis was carried out in 1 x TAE [Tris-acetate-ethylenediaminetetraacetic acid] buffer with a current voltage of 100 V for 16 h at 60°C. The gradient ranged from 30% to 70% denaturant; 100% denaturant corresponds to 7 M urea and 40% foramide. For 18S-rDNA, a 7% polyacrylamide gel was used (7% [w/v] acrylamide, 0.1% [v/v] TEMED [N,N,N′,N′-Tetramethylethan-1,2-diamin], 0.1% [w/v] ammonium persulfate in 1 x TAE [Tris-acetate-ethylenediaminetetraacetic acid] buffer). The gradient ranged from 5 to 40%, the running time was 20 h at 60°C, and a current voltage of 50 V was applied. Bands were visualized by silver staining. Gels were analyzed with GelCompar II (Applied Maths, Sint-Martens-Latem, Belgium). After normalizing the gels and subtracting the background, the Pearson product-moment correlation was calculated. The Pearsons correlation coefficient compares the densiometric curves of the fingerprint. The clustering of

the patterns was calculated using the unweighted pair group method using average linkages (UPGMA).

3.4 Results

3.4.1 Degradation of ^{14}C-labeled wheat straw

The mineralization was observed by measuring the $^{14}CO_2$ emitted from the ^{14}C-labeled wheat straw. As presented in Figures 3.1a and 3.1b, the similarity of the curves from soils treated with ^{137}Cs and ^{90}Sr is remarkable. Almost no differences were observed between the two differently treated soils. The lower and higher contaminated soils, as well as the control soils without the application of radionuclides exhibited equal mineralization rates. However, significant differences in the mineralization curves can be seen between the native and reinoculated soils (Figures 3.1a and 3.1b, black and bright symbols, respectively). Mineralization in native soils started with no lag phase (Figures 3.1a and 3.1b, black lines). The highest mineralization rates were determined within the first two days: between 5.2% and 5.3% in radioactively contaminated soils and control soils (values are the average of triplicates). The mineralization rate decreased continuously until day 20. Here, the mineralization rate was between 0.8% and 0.9% and it remained below 1% until the end of incubation. The mineralization in reinoculated soils also started without a lag phase. The mineralization rate was highest on the second day (between 4.1% and 4.3%). It then decreased until day eight and increased again to and increased again to reach between 3.6% and 3.9% on day 12. From day 12, the mineralization rate decreased continuously until the end of the experiment. After 70 days, the native soils evolved between 41.5% and 43.8%. The reinoculated soils evolved between 47.4% and 49.8% (Figures 3.1a and 3.1b, cumulative evolved $^{14}CO_2$).

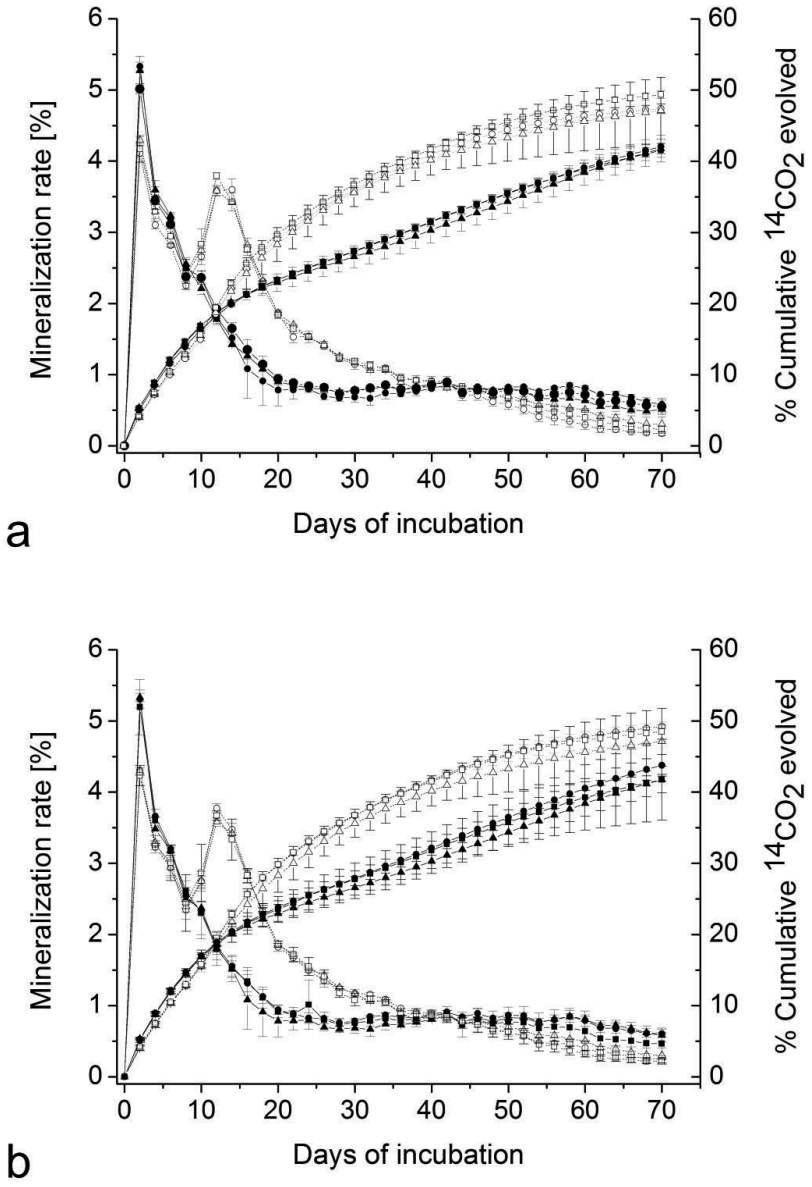


Figure 3.1: Mineralization rate (left ordinate) and cumulative evolved $^{14}CO_2$ (right ordinate) of soil microcosms treated with a: ^{137}Cs or b: ^{90}Sr. Dashed line and bright symbols: sterilized and inoculated soils; solid line and black symbols: native soils, ● and ○: high contamination, ■ and □: low contamination, ▲ and Δ: control soils without radionuclides. The vertical bars refer to standard deviation of triplicates (applied ^{14}C radioactivity 100%).

3.4.2 Community composition

The development of the microbial communities was compared via denaturing gradient gel electrophoresis (DGGE). After 70 days of incubation, soil samples were taken from the microcosms and the DNA was amplified with specific primers for 16S rDNA and 18S rDNA respectively. Based on the results of the mineralization experiment, changes in community structure were not expected in the less contaminated soils. Thus, DNA was compared from control soils and the highest contaminated soils.

Figure 3.2a shows a detail of the 16S rDNA fingerprint of soils incubated for 70 days. The lanes A, B and C represent the soils treated with radiolabeled wheat straw. A was treated with ^{137}Cs (14,800 ± 1,127 Bq g^{-1}, *n = 3*), B was treated with ^{90}Sr (4,510 ± 884 Bq g^{-1}, *n = 3*) and C is a control soil without the application of radionuclides. D represents a further control soil not treated with straw and radionuclides. D acted as a control for changes caused by straw application and incubation conditions. E was the native soil taken from the Merzenhausen field site and was not incubated or treated in any way.

The 16S band pattern is characterized by a few strong bands accompanied by numerous weak bands (Fig. 3.2a). Two strong bands (arrows 3) appear in all lanes except from the reinoculated control soils (lanes D and E). However, the band pattern of native and reinoculated soils can be distinguished, even though these disparities were unclear in cluster analysis. In the reinoculated soils, strong bands appear (arrow 1). These bands do not appear in native soils.

According to bands appearing in native soils (arrow 2a), in reinoculated soils these bands only occur in control soils (arrow 2b, circled). The lanes are clustered into two groups: one small group consisting of the reinoculated control soils without straw application and the Merzenhausen soils, and one group consisting of all the other soils. The Pearson similarity in the former group was at least 57.7%, in the latter group at least 58.2%.

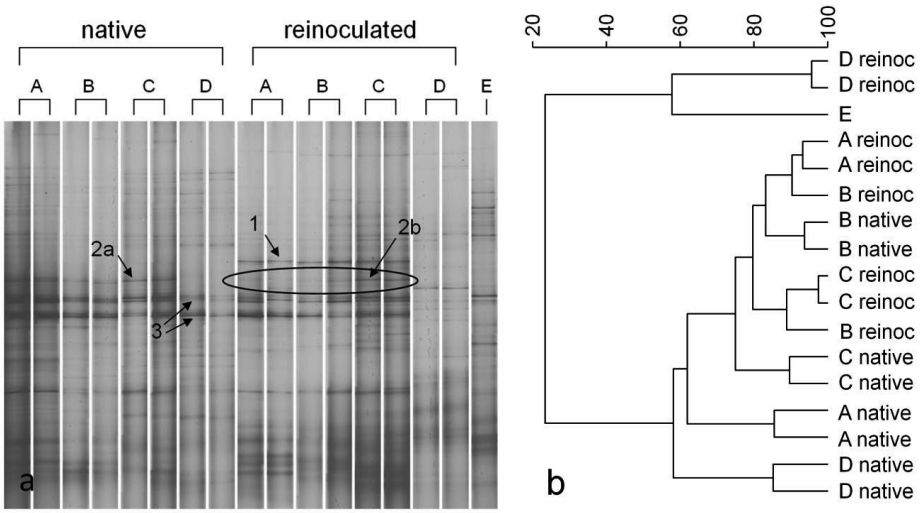


Figure 3.2: a) Detail of 16S rDNA DGGE pattern of radioactively contaminated soils from the microcosm study. Soils A, B and C treated with 2% ^{14}C-labeled wheat straw. A: ^{137}Cs, B: ^{90}Sr, C: control. D: incubated in microcosms: b) Cluster analysis of 16S rDNA DGGE pattern of radioactively contaminated soils from the microcosm study. Clustered with Pearson similarity correlation and UPGMA. Scale represents % similarity.

The 18S DGGE gel is characterized by several strong bands and a fewer weak bands (Figure 3.3a) compared to the bacterial gel. The disparities between native soils and reinoculated soils are remarkable. Only a few bands in native soils are congruent to bands in reinoculated soils (arrows 2, 3 and 4). Within the native soils, the band distribution is homogeneous, while in control soils (lane C), bands appear which are missing in the radioactively contaminated soils (circle 1). However, in reinoculated soils, the band distribution is more heterogeneous. Here, the control soils show the most and the strongest bands. In the soils with radionuclide application some bands are also missing.

In contrast to the 16S rDNA fingerprint, the disparities between native and reinoculated soils are reflected in the cluster analysis (Figure 3.3b). Three groups were clustered: reinoculated control soils without straw application (group 1, Pearson similarity 47.4%), reinoculated soils treated with straw (group 2, Pearson similarity 56.9%), and all the native soils and the control soil from Merzenhausen (group 3, Pearson similarity 62.2%).

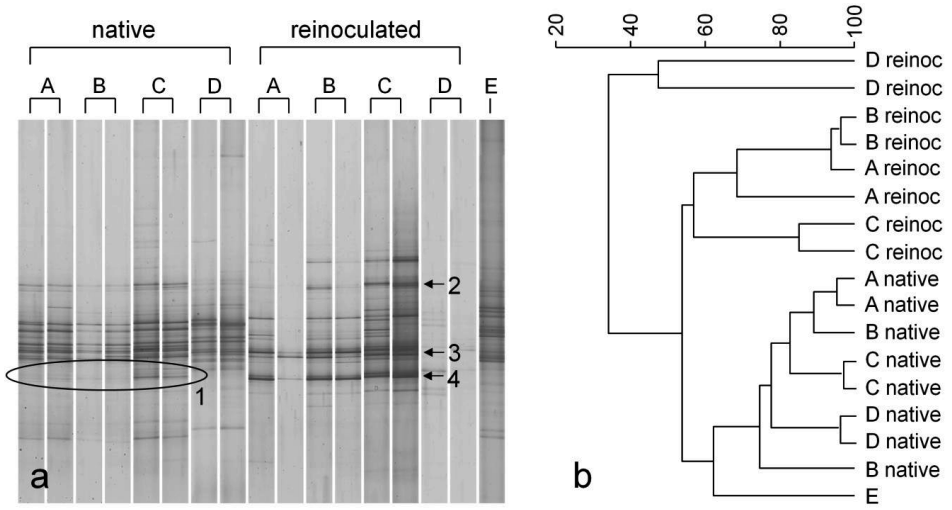


Figure 3.3: a) Detail of 18S rDNA DGGE pattern of radioactively contaminated soils from the microcosm study. Soils A, B and C treated with 2% ^{14}C-labeled wheat straw. A: ^{137}Cs, B: ^{90}Sr, C: control. D: incubated in microcosms: b) Cluster analysis of 16S rDNA DGGE pattern of radioactively contaminated soils from the microcosm study. Clustered with Pearson similarity correlation and UPGMA. Scale represents % similarity.

3.4.3 DOC and TN

The sterilization had remarkable effects on the chemical soil parameters. In comparison to the native soils, sterilized soils contained around 11 times more DOC (sterilized soil: 0.454 ± 0.008 mg g^{-1}; native soil: 0.041 ± 0.003 mg g^{-1}, *n = 3*) and almost 3 times more TN (sterilized soil: 0.089 ± 0.003mg g^{-1}; native soil: 0.034 ± 0.001 mg g^{-1}, *n = 3).* The pH changed from 6.8 in native soils to 7.1 in sterilized soils.

Table 3.1: Radioactivity (in Bq g^{-1}) and dose rate (in Gy) applied to the soil microcosms. The dose rate shows the absorbed dose within one hour (Gy h^{-1}) or within the incubation time of 70 days (Gy 70 d^{-1}). Control soils did not contain ^{137}Cs or ^{90}Sr. ± mean standard deviation of *n = 3*.

	Native			Sterilized and reinoculated		
	Bq g^{-1}	Gy h^{-1}	Gy 70 d^{-1}	Bq g^{-1}	Gy h^{-1}	Gy 70 d^{-1}
^{90}Sr	254 ± 19	$1.8*10^{-4}$	0,3	252 ± 11	$1.8*10^{-4}$	0,3
^{90}Sr	4510 ± 884	$3.2*10^{-3}$	5,5	2875 ± 532	$2.1*10^{-3}$	3,5
^{137}Cs	692 ± 17	$1.2*10^{-4}$	0,2	727 ± 14	$1.3*10^{-4}$	0,2
^{137}Cs	14,800 ± 1127	$2.7*10^{-3}$	4,5	15,033 ± 1101	$2.7*10^{-3}$	4,6
Control	0	0	0	0	0	0

3.5 Discussion

3.5.1 Radiation effects

Prior to incubation, the microcosms were contaminated artificially with various concentrations of cesium nitrate and strontium nitrate. The cumulative doses over 70 days were in the range of 0.2–5.5 Gy, respectively. The radioactivity was applied in accordance with hotspots occurring in 10 km zone around the reactor in Chernobyl. Compared to other experiments (Davis et al., 1956; Eriksen and Emborg, 1978; McNamara et al., 2003; McNamara et al., 2007), the applied radioactivity was relatively low. However, a contamination of (huge) agricultural areas with low doses is more probable than very high doses of several kGy. It is therefore astonishing that the effects of such low doses on ecosystems have not yet been investigated.

We detected no disparities in the straw mineralization between radioactively contaminated soils and control soils. The mineralization rates of native and reinoculated soils were in the same range. The different modes of radioactive contamination (^{137}Cs, ^{90}Sr, high or moderate contamination) had no impact on straw degradation.

In our fungal DGGE patterns, as well as in the bacterial DGGE patterns (Figures 3.2a and 3.2b), population shifts were observed. Lanes of ^{137}Cs- and ^{90}Sr-treated soils did not contain bands which appeared in the corresponding control soils (circles in Figures 3.2a and 3.3a).

Interestingly, this is not consistent for native and reinoculated soils. Bands which were missing in reinoculated soils occurred in native soils in the bacterial gel and vice versa in the fungal DGGE gel. The observed impacts on the communities could not be related to the mineralization of wheat straw. The DGGE method appears limited in this context, detecting only the most abundant microorganisms (Muyzer et al., 1993). However the most abundant microorganisms do not necessarily represent the most dominant microorganisms in function (Torsvik et al., 1990).

There are three possible reasons for the observed effects: the microorganisms repaired DNA damages rapidly, the radiation was insufficient to harm the microorganisms, or the community exhibited a high redundancy of functions (Andren et al., 1995; Ekschmitt et al., 2001; Griffiths et al., 2004). However, the shifts in microbial community structure indicate that the latter reason applies to our soils. Small population shifts were also reported from other authors. Fuma et al. (1998) for example found an inhibited activity of E. coli in microcosms for short periods of time at 100 Gy, and Pitonzo (1999a) found a decrease in the utilization of different carbon sources after short irradiation with a dose rate of minimum 98 Gy. With increasing radiation, the carbon sources utilized by bacteria were simpler in structure. Pitonzo supposed that the limited microbial energy reserves were used for DNA repair rather than for the production of enzymes. The impacts observed in these dose ranges were relatively low. This suggests that the doses applied in our experiment were not enough to hinder soil functions, which are based on microbial activities. The radiation did cause a community shift of the bacterial and fungal population, but the mineralization of wheat straw was not restrained due to redundancy of functions or because straw degrading microorganisms were not affected.

3.5.2 Soil treatment effects

The mineralization of wheat straw started without a lag phase in all applications. This indicates that the microbial community was well adapted to straw degradation and that there was an adequate number of straw degraders (Bauer et al., 2008). For native soils, this can be explained by the fact that the soil samples were collected from an agricultural field site where wheat was planted periodically. The reinoculated soils were also collected from the field site, but they were sterilized via autoclaving and reinoculated with only 1% native soil mass. That means that the microbial soil community in these soils should have been much smaller than in

native soils at the beginning of the incubation. It is surprising that this very small community decomposed almost the same amount of straw as the undisturbed community in native soils. This and the fact that the ^{14}C-labeled wheat straw was not sterilized prior to application suggest that the early stage of mineralization was not only performed by a pristine soil community. Presumably the microorganisms on the straw itself participated much more in the decomposition than expected. When the straw was added, a secondary microbial community was inoculated into the soil microcosms. From Tester (1988), it is known that microorganisms on the straw degrade readily available straw compounds.

Based on our experimental findings we propose the following mechanisms for straw degradation: After depletion of the easily available compounds, such as amino acids and sugars (Aneja et al., 2006), by the microorganisms on the straw, the more recalcitrant and non-water-soluble components, such as lignin-encrusted cellulose (Wang et al., 2004), were degraded by the autochthonous soil microorganisms. More energy is required for microbial enzyme production, which causes a decrease in the release of carbon dioxide. To approve these findings, a supplementary mineralization experiment was conducted (data not published)[3]. Here combusted, autoclaved, autoclaved and reinoculated and native bulk soil respectively were applied with ^{14}C radiolabeled not-sterilized wheat straw. The mineralization was determined with trapping the released $^{14}CO_2$ in NaOH traps and measuring the radioactivity in the LSC. The incubation conditions were the same as in the mineralization experiment described above. In the first two weeks the mineralization rates in the combusted soils and the autoclaved soils have been similar to the mineralization in the native and reinoculated soils, although the former soils did not contain additional soil microorganisms. This suggests that the microbial population, growing on the applied wheat straw, takes a major part on the mineralization of the readily available straw compounds. In contrast, the native and reinoculated soils from the first mineralization experiment had much higher mineralization rates during the same timeframe. Due to an assumed high cell density, limited nutrient availability and a well-developed microbial community, the growth of specialized straw degraders was hindered. In the reinoculated soils, the mineralization rate also decreased having reached its first maximum after two days, presumably due to depletion of the readily available straw compounds (Figures 3.1a and 3.1b). However, after day eight, the

[3] Supplementary experiment see Chapter 3.7

mineralization rate increased again and almost attained the same efficiency at day twelve. In the period between the two peaks, we can therefore assume the establishment of specialized bacteria for straw degradation. Furthermore, it can be suggested that their growth was positively influenced by remaining DOC from the sterilization process and the much lower competition pressure compared to native soils (Liebich et al., 2006). After reaching a sufficient density, the population took over the straw degradation and the carbon dioxide release increased again, as shown by the second peak in the mineralization curves (Figures 3.1a and 3.1b). Marschner et al. (2010) showed recently that the microbial community composition is related to water-extractable C in soil. Fast-growing bacteria species dominate the initial mineralization of organic compounds. After the easily available compounds have been depleted, non-soluble compounds are decomposed and slow-growing microorganisms (fungi and K strategists), which degrade the more recalcitrant compounds, dominate at later stages (Dilly et al., 2004; Blagodatskaya et al., 2010; Marschner et al., 2010). Chronological changes in microbial community structures were not observed because DNA extraction was performed after the incubation period of 70 days. However, alteration of microbial communities caused by straw application and incubation were reflected in the bacterial and fungal DGGE patterns. Native soils treated with wheat straw showed at least one additional strong band compared to soils not treated with straw (Figure 3.3a, lane D). Furthermore, the soils which were not treated with straw showed a different band pattern than the non-incubated soils from the field site (Figures 3.2a and 3.2b, lane E). This underlines that the incubation conditions and the straw influenced the community composition. From Holland and Coleman (1987), it is known that organic matter amendments alter the availability of carbon sources and thus presumably change the soil microbial community structure.

3.6 Conclusions

We applied soil microcosms with various concentrations of ^{137}Cs and ^{90}Sr according to the radioactivity of these radionuclides in the 10 km zone around Chernobyl. The aim was to observe the impact of the radioactive contamination on the degradation of ^{14}C-labeled wheat straw and on the microbial community structure. Slight population shifts were detected in the 16S and 18S rDNA DGGE gels; but the wheat straw degradation was not affected. When soil samples were sterilized and reinoculated with 1% native bulk soil, the DGGE pattern and the mineralization rate changed significantly. From the findings we concluded that the heat

treatment had a major impact on development of the microbial community and the mineralization of the wheat straw. The radioactive contamination plays, even when it is much higher than in the 10 km Chernobyl zone, only a minor role for these soil functions.

3.7 Supplementary experiment: Mineralization of wheat straw and impacts on microbial population in differently heat treated soils[4]

Aim of this supplementary experiment was to analyze the impact of sterilization and reinoculation on the wheat straw degradation and the microbial community structure. ^{14}*C-labeled wheat straw was applied to a) combusted, b) autoclaved, c) autoclaved and reinoculated with 1% native soil and d) native bulk soils, respectively and incubated under the same conditions as the wheat straw mineralization experiment described above.*

Material and Methods

I analyzed the effect of sterilization and reinoculation using soil microcosms with differently heat treated soils. In all cases, an Orthic Luvisol from the field site in Merzenhausen was used. The soils were either kept native, autoclaved three times and reinoculated with 1% [m/m] native soil, autoclaved three times, or ashed for 24 h at 600 °C. In the latter two instances, the soils were not reinoculated with native soil, but were kept sterile until straw application. Each approach was performed in triplicate. The straw application, experimental setup and environmental conditions were identical to the wheat straw mineralization study described in Chapter 3; 100 g soil (dry weight), including 2 g wheat straw; incubated at 20 ± 1 °C, water content 50% WHC, $^{14}CO_2$ *absorbed with 2 ml 2 M NaOH solution, carbondioxide traps changed every second day (until day 18, after that every third or fourth day). For measuring the radioactivity in the LSC the NaOH solution was mixed with 8 ml deionized water and 10 ml scintillation cocktail.*

After 5 weeks, the soil samples were taken and the microbial communities were compared via 16S rDNA DGGE. For details of the DGGE see Chapter 3.3.5.

[4] Chapter 3.7 was not part of the publication "Effects of low-level radioactive soil contamination and sterilization on the degradation of radiolabeled wheat straw"

Results

In the wheat straw microcosm study, the heat treatment caused severe impacts on the mineralization process. To explain this, we examined the mineralization of radiolabeled wheat straw in four differently treated soils. Fig. 3.4 shows the mineralization rate and the cumulative evolved $^{14}CO_2$. The mineralization rate of the ashed soils without the application of additional biotic inoculum (bright triangle) was almost as high as in native soils (inverse black triangle). The maximum mineralization was reached after 4 days for native, ashed and sterile soils, and on day six for the reinoculated soils. Once the maximum had been reached, the mineralization rate of all soils decreased rapidly until day 12. The highest mineralization rate was found for reinoculated soils at 2.4%, followed by native and sterile soils at 1.5%. The mineralization rate of the reinoculated soils on day 12 was 0.8% and it remained in this range until the end of the experiment. After 18 days of incubation, the CO_2 traps were no longer changed every second day, but rather every third or fourth day. Thus, the mineralization rates between day 18 and 38 were interpolated from the given values.

In the 16S rDNA pattern of the differently treated soils, only the bands marked with 1 and 2 are consistent for all lanes (Figure 3.5). In the upper and middle sections of the native soil lanes (lanes F and G), a higher number of strong bands occur. In the autoclaved and ashed soils, the band concentration in the bottom section of the lanes is more distinct (lanes H, I and J). In these soils, numerous bands could not be separated, resulting in a fuzzy smear (bottom). The autoclaved and ashed soils, that were not treated with native soil inoculum (lanes I and J, arrow 4) exhibit bands which cannot be detected in the reinoculated soils. Bands marked with arrows 3 and 5 only appeared in the ashed soils, or were much stronger compared to autoclaved soils. In the cluster analysis, three clusters dominated (Figure 3.5b). The first cluster consisted of the native soils (lane F) and the reinoculated soils (lane H). The Pearson similarity between both soils was at least 66.4%. The second cluster consisted of soils from the Merzenhausen field site (lane G) and reinoculated soils (lane H, Pearson similarity 65.9%), and the third cluster consisted of native soils. The soils from the Merzenhausen field site (lane G) were not incubated or treated with straw.

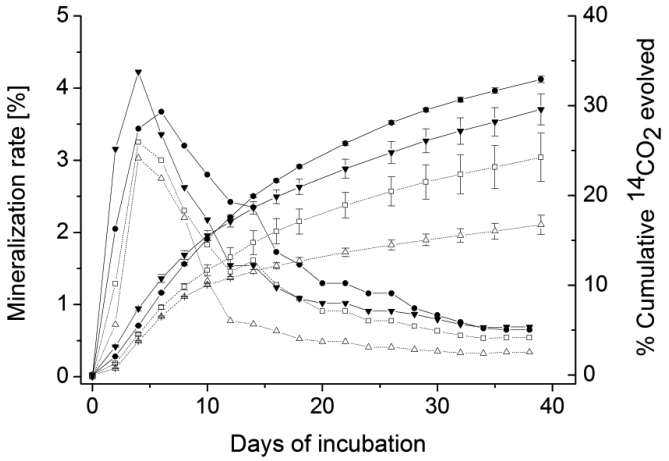


Figure 3.4: Mineralization rate (left ordinate) and percentage of cumulative $^{14}CO_2$ evolved (right ordinate) in different heat-treated soils. ▼: native, ●: threefold autoclaved and reinoculated, □: threefold autoclaved, Δ: ashed. Dashed line: soils without additional application of biotic soil inoculums (applied radioactivity 100%). Due to the irregular exchange of CO_2 traps, values after day 18 were interpolated every second day (only mineralization rate). The vertical bars refer to standard deviation of triplicates.

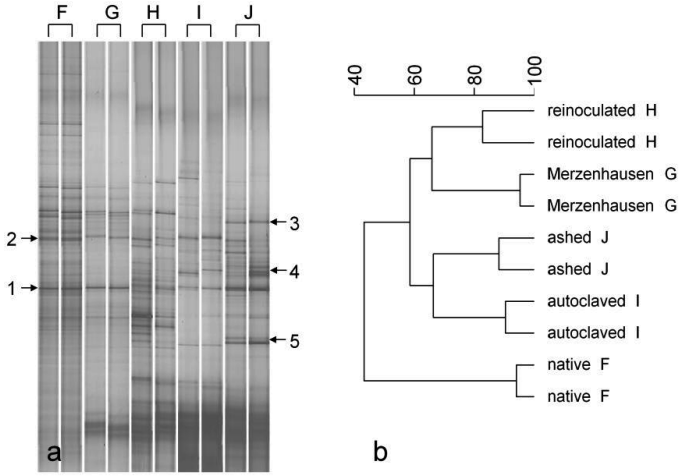


Figure 3.5: a) Detail of 16S rDNA DGGE pattern of heat-treated soils from the supplementary mineralization study. DNA was extracted after 5 weeks of incubation. F native; G soil from Merzenhausen field site, neither incubated nor treated with wheat straw; H autoclaved and reinoculated; I autoclaved but not reinoculated; J ashed but not reinoculated. All soils, except for G treated with 2% [m/m] radiolabeled wheat straw. b) Cluster analysis of 16S rDNA DGGE pattern of heat-treated soils from the supplementary microcosm study. Clustered with Pearson similarity correlation and UPGMA. Scale represents % similarity.

Discussion

In the early stages of straw degradation (Figure 3.4, first 12 days), the mineralization rate of ashed and reinoculated soils was almost as high as in native and reinoculated soils. However, the former two soils did not contain autochthonous soil microbes, but microorganisms imported with the wheat straw. After 12 days, the mineralization rate in ashed soils stagnated presumably due to the depletion of easily available straw compounds. This suggests that the majority of easily available compounds in ashed soils were decomposed by microorganisms on the straw, which agrees with the findings of Tester (1988). He showed that bacteria on straw decompose easily available compounds, but that soil microorganisms are necessary to mineralize more recalcitrant compounds. In native and reinoculated microcosms, pristine soil microorganisms appeared, and thus the mineralization continued more effectively. However, the mineralization in autoclaved soils was surprisingly found to be similarly effective to native soils from day 12 onwards (Figure 3.4). The autoclaved soils were not reinoculated with biotic soil, but in contrast to the ashed soils, the soil organic matter was not removed. This also suggests that the DOC content in soils could be a regulating factor in the degradation of organic matter. Disparities between both soils also are reflected in the DGGE gel (Figure 3.5a, lanes I and J). The ashed soils contain bands, which do not occur in autoclaved soils (arrow 3 and 5).

From day 12 onwards, in the later stage of straw mineralization, reinoculated soils exhibited the highest mineralization rates. On day 14, the mineralization rate was 2.4% compared to 1.5% in native soil, 1.6% in autoclaved soil and only 0.7% in ashed soil. This supports the assumptions from the first experiment, namely that the microbial community in reinoculated soils shifts to straw degrading specialists as a result of sufficient nutrient supply and limited competition. These shifts can be seen in the bacterial band pattern (Figure 3.5). Lanes I and J, which represent the non-reinoculated soils, exhibit a band pattern which is affected by bacteria originating from straw. However in lanes F, G and H, the straw bacteria could not resist and were replaced by recovering soil bacteria originating from biotic soil aliquots. The competition between the straw degraders in native soils and the absence of microorganisms originating from soil in autoclaved soils presumably restrain the straw degradation. In ashed soils, the lack of soil microorganisms and the lack of readily available nutrients could cause the very low mineralization rates.

4 Do Chernobyl-like contaminations with ^{137}Cs and ^{90}Sr affect the microbial community, the fungal biomass and the composition of soil organic matter in soil?[5]

4.1 Objectives

The objectives of the experiment were to investigate the effects of various concentrations of ^{137}Cs or ^{90}Sr on the chemical composition of the soil organic matter and on the development of the fungal biomass. The soils were applied with wheat straw and incubated for 90 days under controlled conditions. The fungal biomass was not determined directly, but compared with the ergosterol content in soil. This was quantified using the HPLC. The chemical composition of the organic matter was determined using the ^{13}C CP/ MAS NMR technique. Despite that alterations in the microbial community structure were compared with help of the 16S rDNA and 18S rDNA DGGE.

4.2 Introduction

Since the isotopes ^{137}Cs and ^{90}Sr are chemically very similar to potassium and calcium, they behave similar in soils. This and the radioactivity make theses radionuclides very hazardous from an ecological point of view. Both isotopes normally do not occur naturally in relevant concentrations (Pyuskyulyan, 2006). However, due to incidents such as atmospheric nuclear weapon tests which continued up to the 1980s, the explosion of the nuclear reactor 4 near Chernobyl (Ukraine) in 1986 or the recent core melt in Fukushima (Japan), these radionuclides were emitted into different layers of the atmosphere. Strong winds and atmospheric currents distributed these radionuclides over short and long distances in varying concentrations (UNSCEAR, 1996). Based on their long half-life (^{137}Cs: 28 and ^{90}Sr: 30 years) and excluding dissipation mechanisms such as lixiviation or plant uptake, theoretically more than half of the ^{137}Cs and ^{90}Sr emitted in the Chernobyl fallout can still be found in the

[5] Adapted from Niedrée, B., Berns, A.E., Vereecken, H., Burauel, P., 2013. Do Chernobyl-like contaminations with ^{137}Cs and ^{90}Sr affect the microbial community, the fungal biomass and the composition of soil organic matter in soil? Journal of Environmental Radioactivity 118, 21-29.

environment. The radioactive fallout from Chernobyl contaminated huge areas all over Europe. The level of radioactive contamination mainly depended on the wind direction and where the clouds released their radioactive load. Large depositions in Western Europe occurred in Southern Germany with ^{137}Cs contaminations of up to 60 kBq m^{-2} (Konopleva et al., 2009). In Austria ^{137}Cs contaminations of 150 kBq m^{-2} (Bossew et al., 1996) and in central Sweden as much as 184 kBq m^{-2} (Rosén et al., 1999) were measured. In Eastern Europe, in Belarus and Ukraine the deposition rates rose to 20,000 kBq m^{-2} (Kashparov et al., 2003) or even 40,000 kBq m^{-2} at hotspots near the reactor (personal communication Dr. Valery Kashparov, 2009, Ukrainian Institute of Agricultural Radiology). Beside regional disparities depending on the wind, other factors affect the amount of radionuclides found in ecosystems. Forest soils usually exhibit higher doses than soils from agricultural areas nearby. This is due to the larger leaf surface in forests leading to an increased leaf adsorption. Furthermore the different cultivation practice in forests infers a lower export of radionuclides as much less plant material is removed on a regular basis. The radionuclides are washed into the soil with the rain. Forest soils usually have a large content of organic matter so the radionuclides remain mobile until they reach the clay containing horizon and are recycled between the plants and the organic soil. On arable land the upper 20 – 40 cm is plowed intensively, which dilutes the radionuclides in what is more or less the plow layer (Konoplev et al., 1993; Kruyts and Delvaux, 2002; Shaw, 2007). In Europe, more than 200,000 km^2 were contaminated by Chernobyl fallout (Kashparov et al., 2003), large parts of which were agricultural areas (Korneev et al., 1988; Alexakhin et al., 2007).

The highest microbial activity is generally found in the upper soil layer due to its high organic matter content, warmer temperatures, changing soil moisture and exchange of gases with the atmosphere, e.g. removal of excess carbon dioxide, which could inhibit growth (Scheffer et al., 2002; Jones et al., 2004). According to the literature, the migration of ^{90}Sr and especially ^{137}Cs into deeper soils layers is rather slow (Zhu and Shaw, 2000; Kruyts and Delvaux, 2002; Konopleva et al., 2009) and both radionuclides usually remain in the upper soil layer for several decades (Drissner et al., 1998; Rafferty et al., 2000). Especially ^{137}Cs is highly absorbed in clay particles. Hence, in contaminated soils microorganisms in the upper soil layers (e.g. the plow layer on agricultural fields) are greatly exposed to radioactivity. Microorganisms are vital key elements in soils as they degrade organic material, such as litter in forests or crop residues in agricultural soils and by doing so return nutrients to the soil and

produce humic substances. The latter are fundamental for fertile soils as they enhance the cation exchange capacity, serve as nutrient carriers and are able to bind heavy metals. Furthermore, humic substances improve the soil structure, the water-holding capacity and influence color of the soil surface and thus the heat balance of soils (Scheffer et al., 2002). Through the excretion of small organic compounds such as polysaccharides, microorganisms add to the aggregation of the different soil components (Griffiths and Burns, 1968; Berns et al., 2008) and hence influence soil functions such as water-holding capacity or cation-exchange capacity. Aside from degrading natural organic matter, the microbial communities are also able to degrade man-made compounds such as pesticides (Burauel and Baßmann, 2005) and are therefore indispensable for an effective remediation of xenobiotics in soil. Changes in the microbial communities thus could have a massive impact on some or all of these soil functions.

To our knowledge, no data are yet available, on how radioactive contaminations in a realistic order of magnitude affect the microbial community structure in soil and hence may have an effect on the degradation of crop residues or the composition of humic substances in soils. McNamara et al. (2007) showed in irradiation experiments with gamma rays that fungi are usually more sensitive to ionizing radiation than bacteria. However, the γ-doses applied were considerably above those occurring in normal environments, even higher than the doses found in the Chernobyl zone.

The objectives of the present study were to determine the impact of Chernobyl-like soil contaminations (i) on the structure of the bacterial and fungal population, (ii) on the fungal biomass and (iii) on the formation of humic substances. To this end we used the 18S rDNA and 16S rDNA DGGE finger-print technique, the quantification of the ergosterol content and 13C-CP/MAS NMR. Ergosterol is an essential ingredient of most fungal cell membranes. It does not exist in animal and plant cells, so it is a useful indicator for the development of fungal biomass (West et al., 1987; Eash et al., 1996).

4.3 Material and methods

4.3.1 Soil characterization

Soil samples were taken randomly from the plow layer of an arable Gleyic Cambisol (FAO) from a field site at Kaldenkirchen–Hülst (51°18'40''N, 6°12'10''E), Germany. The soil texture was determined as sandy loam (sand 73%, silt 23%, clay 4%); C_{org} content was 1.12% and Fe-content 0.7%. The C_{org} and Fe contents of the soil samples were determined on a Leco CHNS-932 analyzer (LECO Corp., St Joseph, MI, USA) and an inductively coupled plasma optical emission spectrometer (ICP-OES, IRIS Intrepid, Thermo Elemental Ltd., Winsford, UK), respectively, at the Central Division of Analytical Chemistry, Forschungszentrum Jülich. The soil was sieved (≤ 2 mm) to remove coarse organic components and stones prior to use.

4.3.2 Soil sterilization and reinoculation

Half of the microcosms were set up with sterilized soil and subsequently reinoculated with a small aliquot of native bulk soil. In the sterilized soils reinoculated with a microbial community, we expected an intensified growth. The subsequent treatment with radionuclides possibly affected the microbial community structure in the early stage of development resulting in disparities compared to native soils in the DGGE analysis. To eliminate fungal spores, sterilization was performed by threefold autoclaving with a mean time of 24 h. After cooling to room temperature, the sterilized soils were reinoculated with 1% [m/m] native, non-sterilized soil and mixed thoroughly. Due to the fact that autoclaving changes the chemical and physical properties in soil (Marschner and Bredow, 2002; McNamara et al., 2003; Berns et al., 2008), the contents of water-soluble carbon (dissolved organic carbon, DOC) and total nitrogen (TN) as well as the pH were determined in some of the samples before and after autoclaving. To this end, 20 g of field moist soil and sterilized soil (dry mass), respectively, were suspended in 160 ml of purified water (MilliQ, Millipore) and shaken for 12 h at 150 rpm on a horizontal shaker. After centrifuging (90 min, 10,000 x g), the supernatant was filtrated (0.45 µm) and analyzed on a TOC-V CPH total organic carbon analyzer (Shimadzu Scientific Instruments, Japan) equipped with a total nitrogen analyzer (TNM-1 unit). The pH values of the supernatants were determined with a S20 SevenEasy pH (Mettler Toledo, Greifensee, Switzerland).

4.3.3 Microcosm setup

The microcosms consisted of 500 ml glass bottles, filled with 196 g soil (dry weight) and 4 g wheat straw. The water content was adjusted to 50% of the water-holding capacity (WHC) (OECD, 2002; Rocha et al., 2006) with purified water (MilliQ, Millipore). To compensate the swelling of the wheat straw, 8 ml deionized water was added as a supplement. The microcosms were incubated at 20 ± 1 °C for three months and checked for water loss by weighing on a weekly basis.

4.3.4 Application of wheat straw and radionuclides

The wheat straw (cultivar: Taifun) contained 39.4% C and 1.31% N. Prior to application the straw was ground to less than 500 µm in an ultracentrifugal mill at 15,000 rpm (Retsch, Germany). Microcosms were set up in different combinations of radionuclide and straw application as shown in Table 1.

The radionuclides ^{137}Cs and ^{90}Sr were supplied as nitrate salts (Ritverc, St. Petersburg, Russia). The pure radionuclides were dissolved in deionized water and added to previously ashed soil aliquots (5 g). Prior ashing was applied to remove hydrophobic humic substances to allow the absorption of water without lump formation. After drying, the soil aliquots were ground, added to the soil microcosms and thoroughly mixed in an end-over-end shaker for 45 min. The radioactivity and dose rates were determined at the Central Division of Research Reactors and Nuclear Service at Forschungszentrum Jülich. The radioactivity of cesium was determined with n-type high-purity germanium detectors (Eurisys Mesures, France). Three aliquots were taken randomly from each ^{137}Cs-treated microcosm and measured as one pooled sample. The ^{90}Sr activity was measured with the beta counter FHT 770R (Thermo Electron Corporation, Waltham, Massachusetts, USA). Due to the high sensitivity of the detector, the ^{90}Sr samples had to be rather small. Three aliquots were measured to avoid inaccuracies caused by local hot spots. The absorbed dose [Gy; Gray] was calculated using a particle track calculation and a Monte Carlo simulation (MCNP5, Los Alamos National Laboratory, USA). The radioactivity applied in the microcosms ranged from 10,347 Bq g^{-1} (^{90}Sr) to 15,900 Bq g^{-1} (^{137}Cs). This corresponds approximately to 30 to 50-fold of the hotspots in the exclusion zone near Chernobyl, which is 40 MBq m^{-2}. Assuming a soil density of 1.3 g cm^{-3} and a

homogeneous distribution of the radionuclides in the upper 10 cm, the contamination relative to the soil mass in the hotspots is about 307 Bq g^{-1}.

Table 4.1: Radioactivity (in Bq g^{-1}) and dose rate (in Gy h^{-1}) applied to the soil microcosms. Control soils not treated with ^{137}Cs or ^{90}Sr (Ctrl*) or not treated with radionuclides and wheat straw (Ctrl**). Cesium samples were measured as one pooled sample, strontium samples were measured in triplicate (± mean standard deviation of *n = 3*). ×: applied, –: not applied with wheat straw or radionuclides, respectively.

	Native		Sterilized and reinoculated		Wheat straw
	Bq g^{-1}	Gy h^{-1}	Bq g^{-1}	Gy h^{-1}	
^{90}Sr	10,347 ±	$7.45*10^{-3}$	11,167 ±	$5.31*10^{-4}$	×
^{137}Cs	14,700	$2.6*10^{-3}$	15,900	$2.9*10^{-3}$	×
Ctrl*	–	–	–	–	×
Ctrl**	–	–	–	–	–

4.3.5 Denaturing gradient gel electrophoresis

After an incubation time of 13 weeks, the DNA was extracted with the extraction kit "FastDNA Spin Kit for Soil, BIO101" from Qbiogene, Carlsbad, USA. The amplification was performed with a Thermocycler (iCycler IQ from Bio Rad Laboratories, USA) running the PCR program as follows: activation of the hotstart polymerase for 10 min at 95 °C, polymerase chain reaction (PCR) in 35 cycles, each cycle consisting of 1 min at 94 °C, 1 min at 54 °C and 1 min at 72 °C, followed by a single period of 10 min at 72 °C. The chemicals for the amplification mixture were purchased from Thermo Scientific (Waltham, Massachusetts). The 16S amplification mixture consisted of: 5 µl reaction buffer (750 mM Tris-HCl, pH 8.8 at 25 °C, 200 mM $(NH_4)_2SO_4$, 0.1% (v/v) Tween, 15 mM $MgCl_2$), 2.5 µl DMSO, 2 U hotstart polymerase and 20 pmol of each primer. The 18S amplification mixture consisted of 5 µl reaction buffer (750 mM Tris-HCl, pH 8.8 at 25 °C, 200 mM $(NH_4)_2SO_4$, 0.1% (v/v) Tween, 15 mM $MgCl_2$), 3 µl 25 mM $MgCl_2$, 1.5 µl DMSO, 2 U hotstart polymerase and 14 pmol of primer NS1 and 8 pmol of primer GCFung. The primers for eubacterial 16S rDNA genes were 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'). The primers for fungal 18S rDNA genes were NS1 (5'-GTA GTC ATA TGC TTG TCT C -3') and GCFung (CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC CAT TCC CCG TTA CCC GTT G-3'). Both are known to amplify fragments of 350 bp in length (Muyzer et al., 1993; May et al., 2001). Primer GCFung included a 40-base GC clamp for enhancing the melting stability of the PCR products in the DGGE (Ferris et al., 1997). The primers were purchased from Eurofins MWG Operon (Ebersberg, Germany).

The Denaturing gradient gel electrophoresis was carried out in a Dcode Universal Mutation Detection System (BioRad, USA) in 1 x TAE [Tris-acetate-ethylenediaminetetraacetic acid] buffer with a current voltage of 100 V for 16 h (16S) or with a current voltage of 60 V for 20 h (18S). In the case of 16S rDNA, the gels consisted of a 6% polyacrylamide gel (6% [w/v] acrylamide, 0.1% [v/v] TEMED [N,N,N′,N′-tetramethylethan-1,2-diamine], 0.1% [w/v] ammonium persulfate), the gradient ranged from 30% to 70% denaturant; 100% denaturant corresponds to 7 M urea and 40% formamide. In the case of 18S rDNA, a 7% polyacrylamide gel was used (7% [w/v] acrylamide, 0.1% [v/v] TEMED [N,N,N′,N′-Tetramethylethan-1,2-diamin], 0.1% [w/v] ammonium persulfate in 1 x TAE [Tris-acetate-ethylenediaminetetraacetic acid] buffer). The gradient ranged from 5 to 40%.

Bands were stained with silver nitrate. Gels were analyzed with GelCompar II Version 3.0 (Applied Maths, Sint-Martens-Latem, Belgium). Prior to calculating the similarity by the unweighted pair group method with arithmetic mean (UPGMA) and Persons product-moment correlation coefficient, the band patterns were normalized and the background was subtracted by the rolling ball method.

4.3.6 Extraction and quantification of ergosterol

Once a week the ergosterol was extracted from soil aliquots according to a simplified method of Eash et al. (1996), which was modified in some points after Liebich et al. (2003):

3 g soil, suspended in 18 ml cold methanol (-20 °C) and 6 ml KOH (4% KOH (w/v) in 95% ethanol), was placed in pressure-resistant centrifugation tubes and homogenized on a vortex shaker for a few seconds. After sonification for 1 min, the samples were incubated in a water bath at 85 °C for 30 min. After cooling to room temperature, 5 ml deionized water (MilliQ)

was added and the samples were mixed on the vortex shaker again for a few seconds. The extraction mixture was filtered through a 0.45 µm membrane filter (cellulose acetate filter porafil CA, Macherey und Nagel, Düren, Germany) and flushed with 5 ml methanol. The filtrate was transferred to sterile 50 ml reaction tubes and extracted three times with 6 ml n-hexane. Between each extraction step the reaction tube was shaken for 2 min. The hexane phases were removed with a pipette and transferred into a 20 ml glass vial. The hexane was evaporated under nitrogen flow and with an external heat supply (60 °C). 5 ml methanol was added and the samples were measured directly in the HPLC. If measurements were first made on day later, the samples were stored in the dark in a freezer (-20 °C). To prevent decay caused by ultraviolet radiation, all extraction steps were performed in a darkened laboratory. The chemicals used were HPLC grade.

Ergosterol quantification was done in an HPLC-UV/VIS at room temperature on a LiChrospher 60RP Select B 5µ-column and a precolumn filled with the same material. To separate the matrix from the ergosterol the eluent and the flow rate alternated. Beginning with 1 ml min^{-1} acetonitrile, the eluent was changed after 5 min to 0.5 ml min^{-1} methanol for 15 min and then back to 1 ml min^{-1} acetonitrile. The ergosterol retention time was approximately 15 min. The measurement was performed with a diode array detector (Dionex UV-D340s) at 282 nm. The injection volume was 100 µl. The limit of detection (LOD) was 0.025 mg l^{-1}; limit of quantification (LOQ) was 0.1 mg l^{-1}. The mean values of the two measured replicates are shown in Fig. 2.

4.3.7 NMR sample preparation

To eliminate paramagnetic elements such as iron, the samples were treated with hydrofluoric acid as described in Berns et al. (2008). Briefly, 3 g dry soil was shaken with 30 ml 2% HF (v/v) for 24 hours. The samples were then centrifuged for 1 hour at 48,000 x g and the supernatant was discarded. This procedure was repeated five times. The samples were subsequently washed with 30 ml of distilled water to remove residual hydrofluoric acid and then freeze-dried.

4.3.8 Solid state NMR spectroscopy

General conditions. Spectra were obtained on a 7.05 T Varian INOVA^{TM} Unity (Varian Inc., Palo Alto, CA, USA) equipped with an HX Apex probe (75.4 MHz for ^{13}C). The temperature

was set to 25 ± 0.1 °C and magic angle spinning was carried out at 7500 ± 1 Hz. Samples were packed in zirconium Pencil® rotors (diameter 6 mm) with Vespel® drive tips and custom-made boron nitride inlays to position the samples in the homogeneous region of the sampling coil (Berns and Conte, 2010). All spectra were recorded with a sweep width of 25 kHz and an acquisition time of 20 ms. Decoupling was done with a SPINAL sequence (4.51° phase; 11 µs pulse width).

$^{13}C\{^1H\}$Cross-Polarization. A 90° ^1H pulse of 3.6 µs was applied followed by a cross-polarization (CP) spin lock pulse of 1 ms. During CP, the radio-frequency field applied to ^{13}C was kept constant, while the ^1H field was set up with an ascending ramp to account for inhomogeneities of the Hartmann-Hahn condition (Berns and Conte, 2011).

Longitudinal ^1H relaxation (T_1H). The T_1H relaxation was determined with an inversion-recovery sequence, which consisted of inserting a 180° pulse on ^1H and a recovery time *t* before the regular CP sequence. The recovery time *t* was incremented from 0 to 1 s in 10 steps. Depending on the available spectrometer time, 16*k* or 32*k* transients (nt) with a recycle delay (d1) of 1.5 s were acquired.

Evaluation. The arrayed experiments were evaluated with MestreNova Version 6.2.1 (MestreLab, Santiago de Compostela, Spain). Fourier transformation was done with a 50 Hz line broadening and baseline correction through a manual multipoint correction. The spectral shift regions were integrated according to Wilson (1987): region I, 0-43 ppm (alkyl C); region II, 43-90 ppm (substituted O- and N-alkyls); region III, 90-110 ppm (anomeric C), region IV, 110-160 ppm (aromatic C) and region V, 160-190 ppm (carboxylic C) (Wilson, 1987). The relaxation curves were fitted with a stretched exponential (Eq. 1) in OriginPro 8G SR6 (Version 8.0988, OriginLab Corporation, Northampton, MA, USA):

$$I = I_0 \left(1 - 2 \exp[-(t/T_1H)^b]\right) \qquad \text{(Eq. 1)}$$

Where $I_0 \equiv I$ ($t=\infty$), $1/T_1H$ is the relaxation rate, T_1H is the relaxation time and the stretching exponent b is in the range $0<b<1$. The errors reported result from the fitting procedure.

4.4 Results

4.4.1 Microbial community

The development of the microbial community structure was compared with the DGGE fingerprint technique. After three months of incubation, the DNA was extracted and amplified with primers specific for 16S and 18S rDNA. Native soils were treated with 14,700 Bq g^{-1} ^{137}Cs or 10,347 ± 532 Bq g^{-1} ^{90}Sr. 15,900 Bq g^{-1} ^{137}Cs or 11,167 ± 1328 Bq g^{-1} ^{90}Sr was applied to the reinoculated soils. Ctrl* represents the control soils treated with wheat straw, but not treated with radionuclides. Ctrl** soils were treated with neither straw nor radionuclides (Table 4.1).

Fig.4.1 shows a detail of the bacterial DGGE gel. A clear-cut differentiation of the DGGE bands is hindered by the remarkably high density of the latter. The native and the sterilized and reinoculated soils produced a different band distribution in the DGGE gels. The Pearson similarity between the two groups is 14%. Within the group of native soils, the Pearson similarity is at least 26%. Except for the Ctrl** lanes, the similarity is 59%. Within the reinoculated soils, the Pearson similarity is at least 56%. The reinoculated Ctrl** lanes form a unique group and have and have only 11% to the other lanes. In control soils (Ctrl*) strong bands were either absent (arrow 3) or present (arrow 1), which is contrary to the corresponding positions in the radionuclide-treated soils (Fig. 4.1a). The Ctrl** lanes exhibit a deviating band distribution. Strong disparities between the straw-treated soils are highlighted by arrows 4 and 2. In the fungal band pattern (Fig. 4.1b), the density of bands is much lower compared to the bacterial DGGE gel. The band distribution of the native soils differs distinctly from that of reinoculated soils. Except for Ctrl** soils, the band distributions of all lanes in the native soil group are congruent to each other. The Pearson similarity of the native soils is, excluding Ctrl**, at least 84%. Within the group of the reinoculated soils, the Pearson similarity between the lanes except for the Ctrl** soils, is at least 85%. The control soils (Ctrl* and Ctrl**) exhibit bands which are absent at the corresponding position in radionuclide-treated soils (Fig. 4.1b, arrow 6). At the positions of arrows 5 and 7 in Ctrl** soils, the bands are absent which appear in the other soil lanes at the corresponding position.

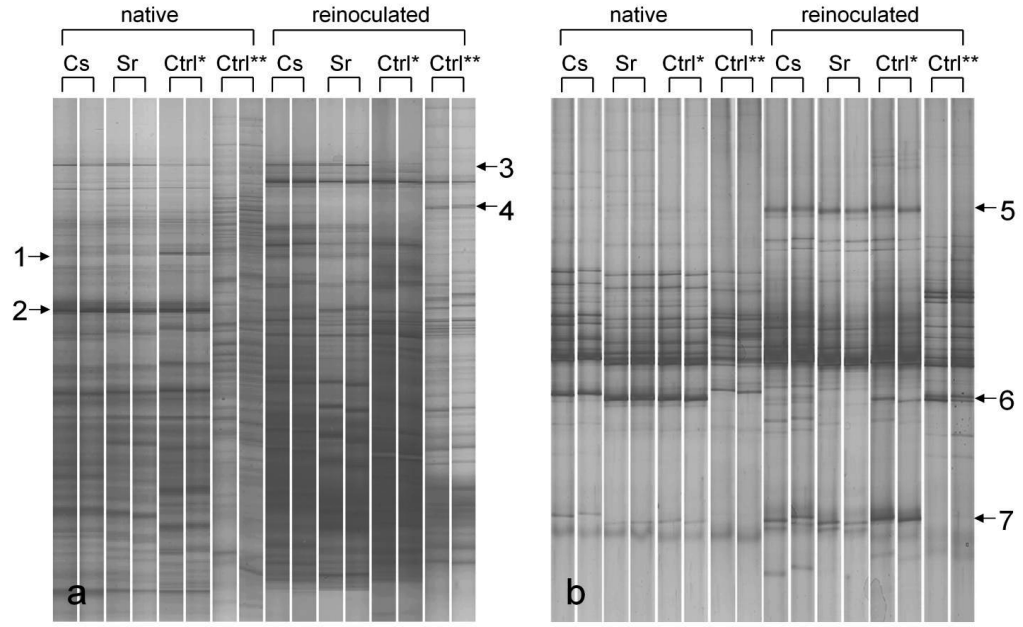


Figure 4.1: Detail of a) the bacterial 16S rDNA and b) fungal 18S rDNA DGGE pattern of soils from the microcosm study. Cs, Sr and Ctrl* treated with 2% wheat straw, Cs and Sr treated with ^{137}Cs and ^{90}Sr, Ctrl*: control with wheat straw, Ctrl**: control without wheat straw.

4.4.2 Development of the fungal biomass

Relative changes in the fungal biomass were determined by quantifying the ergosterol content using HPLC. One day after the start of incubation, the ergosterol content in the native soils is between 2.1 and 4.3 µg g^{-1} (Fig. 4.2, week 0). Except for the native control soil without straw treatment (Fig. 4.2, black stars), the ergosterol content in the reinoculated soils is lower (0.4 - 1.2 µg g^{-1}) than in the native soils. Up to week three, the ergosterol content of the native and reinoculated soils decreases. The ergosterol content in the native soils shows a fluctuating course; it increases with a maximum at week 5 and decreases again up to week 10, whereas the ergosterol content of all native treatments persists in the range lower than 0.6 µg g^{-1}. At week 8, the ergosterol content in the native control soils reaches 1.7 µg g^{-1}, whereas the other native soils exhibit only 0.9 µg g^{-1} at maximum. Between week 10 and the last sampling at week 13, no samples were taken from the soil microcosms. During that period the ergosterol

content in native soils increases slightly up to 0.7 µg g^{-1}, the native Ctrl* soil even increases up to 1.5 µg g^{-1}. The reinoculated soils remain on a constant level between 0.1 and 0.5 µg g^{-1} up to the end of incubation. The ergosterol content in the Ctrl** soils (asterisks, no straw treatment) is lower than the corresponding values from the native and reinoculated soils and does not fluctuate so intensively. From the third week on, the values for the ergosterol content of the reinoculated soils are below the limit of quantification of the HPLC.

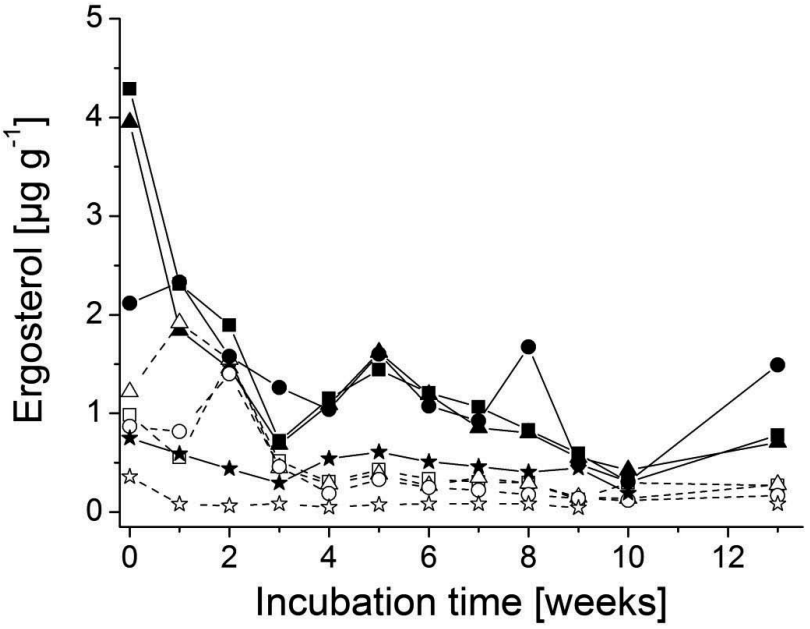


Figure 4.2: Ergosterol content [µg g^{-1}] in soils treated with ^{137}Cs [■ and □], ^{90}Sr [▲ and Δ] or control soils with [● and ○] and without [★ and ☆] straw application. Black line and symbols: native soil; dashed line and empty symbols: sterilized and reinoculated soil. First soil sampling at week 0 was conducted one day after beginning of incubation.

4.4.3 DOC, TN and pH

Before autoclaving the DOC content was 0.059 ± 0.002 mg g^{-1} (*n = 3*) and the TN was 0.019 ± 0.001 mg g^{-1} (*n = 3*), pH was 6.2. After autoclaving the DOC increased to 0.547 ± 0.035 mg

g^{-1} (*n = 3*) and the TN increased to 0.106 ± 0.001 mg g^{-1} (*n = 3*). The pH was not affected and remained at pH 6.2.

4.4.4 Composition of the soil organic matter

Figure 4.4 presents ^{13}C CP/ MAS NMR spectra of samples taken from native and reinoculated soils. All the spectra are typical soil spectra with dominant shift regions I (0-43 ppm), II (43-90 ppm) and V (160-190 ppm). In the native soil samples (Fig. 4.3a), only small and insignificant changes can be observed. The spectrum of the native soil incubated with 14,700 Bq g^{-1} of ^{137}Cs displayed a slightly lower aromatic region IV after 9 weeks. After the same incubation time the aliphatic region (region I) in the native soil incubated with 10,300 Bq g^{-1} of ^{90}Sr appeared somewhat slenderized. The spectra of the sterilized and reinoculated soils (Fig. 4.3b) showed a few more differences. The aliphatic region increased in all spectra after 9 weeks of incubation. In the case of soils treated with ^{137}Cs and ^{90}Sr the shape of the aliphatic signal also broadened. The first half of the carbohydrate region showed a higher and more resolved methoxy peak at 56 ppm after 9 weeks. Also the carboxyl region V increased with time.

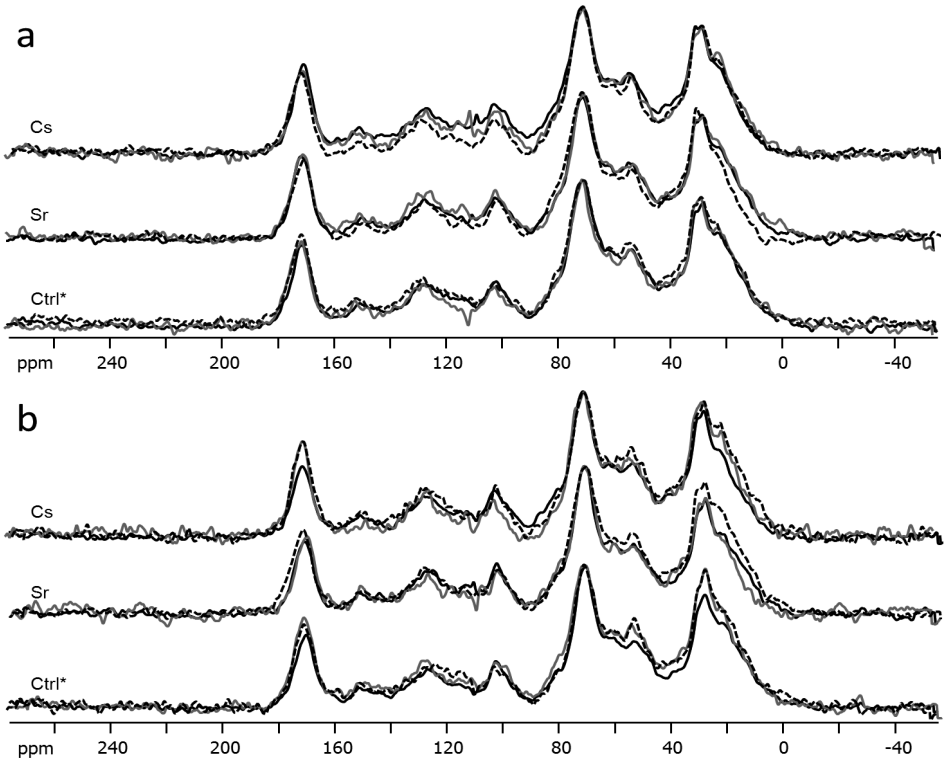


Figure 4.3: a) ^{13}C CP/MAS spectra of the native soils treated with straw sampled one week (black line), five weeks (gray line) and nine weeks (dashed line) after application of the radionuclides. b) ^{13}C CP/MAS spectra of the reinoculated soils treated with straw sampled one week (black line), five weeks (gray line) and nine weeks (dashed line) after application of the radionuclides. All spectra are graphically normalized to the signal at 72 ppm.

Figure 4.4 displays the proton relaxation times T_1H (Fig. 4.4a) as determined by a stretched exponential fit and the respective stretching factors (Fig. 4.4b). The behavior of the relaxation times did not show a clear trend. The increase in T_1H observed in the 9th week for most of the samples and chemical shift regions was also seen in the native control and partly also in the sterilized and reinoculated control. Within one sample, the longest relaxation times were always detected in regions I to III. The stretching factors b were all located between 0.5 and 1 and no significant differences could be identified among them.

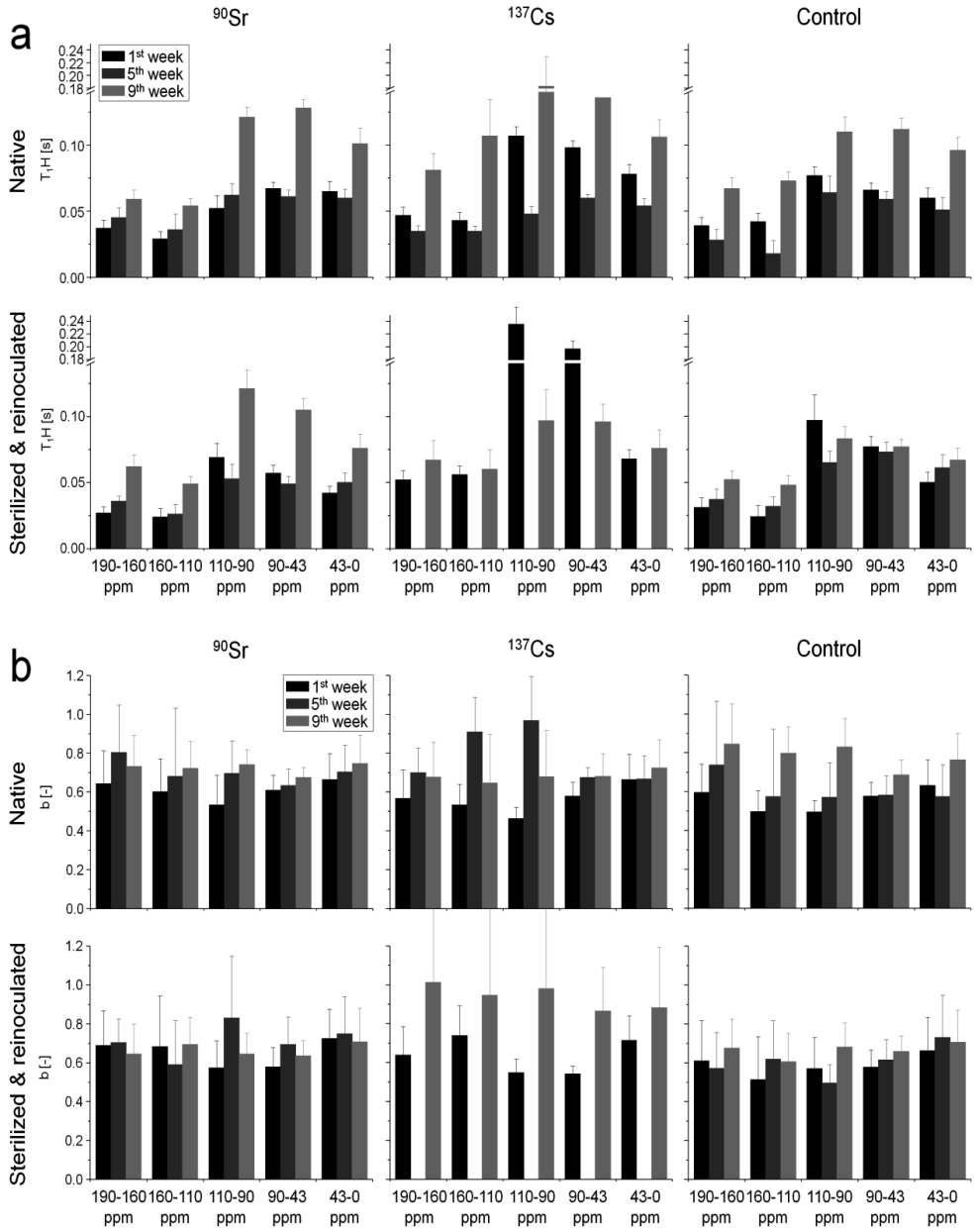


Figure 4.4: a) Longitudinal relaxation times (T_1H) in native and sterilized soils treated with straw sampled one week (black), five weeks (dark gray) and nine weeks (light gray) after application of the radionuclides. b) Corresponding stretching factors (b) of the stretched exponential fitting (see Eq. 1).

4.5 Discussion

4.5.1 Development of the microbial community

The distinct disparities between the microbial population in native soils and soils which were sterilized and reinoculated (Fig. 4.1a and 4.1b) were presumably caused by changes of the nutrition support (e.g. the content of DOC and TN), which was pointed out in a previous publication (Niedrée et al., 2012). The high temperature (121 °C) and pressure (2 bar) during autoclaving affects the physical and chemical structure of the soil and liberates dissolved organic carbon (DOC) (Marschner and Bredow, 2002; McNamara et al., 2003; Berns et al., 2008). Due to the sterilization procedure, the content of DOC that is water-soluble, and thus readily available for microorganisms, increases 9-fold and the total nitrogen content increases 5-fold. This circumstance and the lack of pressure from an established microbial community presumably allow the growth of species that do not usually constitute a dominating population in these soils.

Population shifts caused by the applied radionuclides could be seen in the bacterial as well as in the fungal DGGE patterns. In the bacterial DGGE gel (Fig. 4.1a), there were two different behaviors of bacterial strains. At the position marked with arrow 3, the bacterial species seem to benefit from radiation. The bands occur much more strongly in the contaminated soils compared to the control soils. This pattern is coincident for the native as well as the reinoculated soils, although the population shift is less pronounced in the native soils. This behavior can be explained by interspecific competition. The contamination with ^{137}Cs or ^{90}Sr inhibits or kills specified species and the corresponding competitors benefit from larger nutrient support and less competition. A similar effect was shown by El-Sayed and Ghanem (2009) and McNamara et al. (2007), although the dose rates of the external gamma sources they used, were much higher, around 4 kGy and 10 kGy, respectively. However, at position 1 (Fig. 4.1a) the radiation presumably inhibits the bacterial growth in the native soils. In contrast to the control soils, the contaminated soils do not exhibit bands at that position.

The population shifts displayed at arrow 6 (Fig. 4.1b) seem to be the only visible impact of the radiation on the fungal community structure. In the reinoculated control soils (Ctrl*), bands occur which are not found at the same position in the contaminated soil lanes. This effect is in line with the results from the ergosterol measurements. After 13 weeks of incubation, the fungal biomass of the control soils (Ctrl*) increases and reaches almost double

that of the contaminated soils (Fig. 4.2). At that time, i.e. directly after the last soil sampling for ergosterol measurement, the DNA was extracted and prepared for the DGGE analyses. Possibly at that late stage of straw degradation, some fungal strains recover and mineralize the remaining recalcitrant compounds, which are not available for bacteria. This process may be hindered in the radioactively contaminated soils. However, it is not possible to draw a conclusion from the bands on DGGE gels with respect to species functions and the rDNA DGGE fingerprinting technique is limited in describing the whole microbial community. Only the most abundant species appear as bands, less abundant ones fail to appear (Muyzer et al., 1993).

The straw application seems to have an immense impact on the bacterial and the fungal community. The similarity between the Ctrl** soils and the straw-treated ones, particularly in the bacterial band pattern, is quite small (Fig. 4.1a and 4.1b). This effect is also reflected in the ergosterol quantification, where the straw-treated soils exhibit a much larger fungal biomass than those not treated with straw (Fig.4.2).

4.5.2 Development of the fungal biomass

The growth of the fungal biomass in the microcosms was estimated with help of the ergosterol content. Studies have shown that the ergosterol production correlates to the decay of organic material (Gessner and Schwoerbel, 1991). However, quantification of the biomass still seems to be problematic by this method, because the ergosterol content varies depending on the fungal species and their metabolic states (Bermingham et al., 1995; Stahl and Parkin, 1996). We therefore decided only to compare the relative changes of the fungal biomass between radioactively contaminated and non-contaminated soils.

In the first two weeks of incubation the ergosterol content in the native and the reinoculated soils is quite high compared to the ergosterol content several weeks later (Fig. 4.2). Ergosterol measurements began one day after the beginning of incubation, so no data for day 0 are available. However, due to similar conditions at the beginning and thorough mixing prior to incubation, we assume ergosterol contents in all microcosms comparable to the Ctrl** soils, namely between 0.4 and 0.8 $\mu g\ g^{-1}$ (Fig. 4.2, stars). Here the conditions never change (no straw, no radionuclides) and the ergosterol content remains constant for the whole incubation time.

The high ergosterol concentration after one day and the strong variations in the native soil group presumably result from an intense fungal growth. The lack of a lag phase indicates that the microbial population in the agricultural soils was adapted to regular wheat straw application due to crop residues (Bauer et al., 2008).

In both the native and the reinoculated soils, the rapid decrease of the ergosterol content between the first and the third week is remarkable. The values decrease from the maxima 4.3 µg g^{-1} (native) and 1.2 µg g^{-1} (reinoculated) at the beginning of incubation to lower than 1 µg g^{-1} at week three. Two circumstances could be responsible for this: the periodical disturbance of the soil microcosms due to extraction of soil samples and the two-phase degrading behavior of wheat straw. Bardgett et al. (2001) concluded that in disturbed soils bacteria dominate in the decomposer food web, whereas in undisturbed soils fungi are more abundant. The periodical sampling of approximately 3 g soil possibly disturbed the growth of the fungal community and inhibited a larger formation of ergosterol. This assumption is supported by the increase of the ergosterol content after week 10 (Fig. 4.2). Between the 10^{th} and the 13^{th} week no soil aliquots were taken and the microcosms remained undisturbed for three weeks. Probably the fungal population was able to recover and the ergosterol content in soil increased. Despite that, fungi are known to dominate the second phase of straw degradation. After depletion of the readily available and water-solvent organic compounds by the bacteria, the remaining more recalcitrant compounds were decomposed by fungi and K-strategists (Dilly et al., 2004; Blagodatskaya et al., 2010; Marschner et al., 2010).

Effects possibly caused by the ionizing radiation can be seen at week 8 and 13 in the native soils. The Ctrl* soils exhibit significantly larger ergosterol contents than the radioactively contaminated soils. It is remarkably that this effect is not coincident for all sampling dates. General, knowledge is sparse about the effects of ionizing radiation on fungi, particularly at low doses. From fungal species isolated from radioactively contaminated areas it is known that they can leverage from the ionizing radiation in a kind of radiotropism (Zhdanova et al., 2004; Tugay et al., 2006). However, this behavior is presumably peculiar to fungi which are adapted to radioactive contaminations. That these effects also occur in previously uncontaminated soils has not been observed so far.

According to the literature, the doses usually affecting fungal species seem to be relatively high. The lowest doses impacting fungi are described by Kimura (2006). The gamma and X-ray irradiation of isolated yeast cells led to an up- and down-regulation of functional genes.

The applied acute dose was 50 Gy. In contrast, the calculated dose rate in our experiment was $7.45*10^{-3}$ Gy h^{-1}; in three months 16 Gy was accumulated. Compared to the literature, this dose seems to be too low to affect fungal species. Therefore we assume that the observed effects at week 8 and 13 were presumably not induced by the applied radionuclides.

4.5.3 Composition of the soil organic matter

The low dose rate, compared to dose rates applied for sterilization purposes, was not expected to have any immediate effects on the chemical composition of the soil organic matter (SOM) (Berns et al., 2008). Conversely, we expected the applied dose rates to have an impact on the microbial community structure with possible indirect effects on the degradation and alteration of the organic compounds. However, as described above, the NMR spectra revealed only marginal changes during the incubation. Although the necessary HF treatment might remove soluble components, the content of the latter is proportionately low and hence the loss of dissolved organic matter (DOM) is usually negligible (Knicker and Luedemann, 1995; Berns et al., 2008).

The degradation of wheat straw is typically described in two degradation stages: in the first stage the water-soluble and readily available compounds are degraded and in the second stage non water soluble compounds are utilized by microbial species. The most important decomposition occurs in the first stage and usually takes a few days (Knicker and Luedemann, 1995; Blanco and Almendros, 1997). This stage is characterized by a decrease of the carbohydrates (NMR-shift region II, 43-90 ppm) (Knicker and Luedemann, 1995). However, the NMR spectra did not show a major loss of carbohydrates, neither in the native soils nor in the sterilized and reinoculated soils. This may be due to several reasons. The first sampling took place one week after the start of incubation. Hence, the first stage of straw decomposition was probably not captured. Furthermore, the HF treatment and the subsequent washings most likely removed solid straw residues as a floating fraction, which caused the loss of part of the carbohydrate fraction.

As reported above, autoclaving and reinoculation permanently affects the composition of the microbial communities. Compared to the native soils, the spectra of the sterilized/reinoculated soils show a few changes during incubation with radionuclides such as increases in the aliphatic region I (0-43 ppm) and the carboxyl region V (160-190 ppm). However, the

changes were also noted in the control samples, which indicate that these changes were mainly due to the sterilization process and the subsequent recovery. On the whole, the spectra did not differ greatly from the spectra of the native soils and the process of soil organic matter turnover appeared not to be affected on the long term. The same holds true for the effect caused by the radionuclide treatments. The ^{13}C-spectra gave no indication for changes caused by the radionuclides applied.

Relaxation time measurements were performed to monitor changes in the relaxation behavior of the different chemical structures (Fig 4.4a). As the organic matter in soil is composed of a large range of compounds, each chemical shift region in the NMR spectrum corresponds to a multitude of connatural structures, which, although having similar electronic shieldings (i.e. chemical shift), may well differ in their relaxation behavior due to different degrees of crystallinity (e.g. cellulose) or different mobility (e.g. long vs. short chains). The stretched exponential fit, however, provides only an averaged relaxation time. A shift from shorter to longer relaxation times may hence either be due to an overall change in mobility of the respective chemical structures or, more likely, to a residual accumulation of the slower relaxing components. This slight tendency to longer relaxation times was, however, also seen in the control samples and was most likely not related to the presence of the radionuclides.

The stretching factors b (Fig. 4.4b) all ranged from 0.5 to 1 with a slight, but not significant, tendency to increase over time. According to Bakhmutov (2012), stretching factors ranging from 0.5 to 1 can either be due to "a 'diffusion-limited' relaxation mechanism via random paramagnetic impurities" or "a superposition of several mono-exponential decays in the presence of *smooth relaxation-time distribution*". Both explanations may be applicable to soil samples. The HF treatment removed the major fraction of the mineral paramagnetic compounds, such as iron or manganese. However, a second source of paramagnetism may originate from organic radicals. Hence, the relaxation mechanism through paramagnetic impurities is most likely not completely repressed. As described above, each chemical shift region is composed of several chemically related compounds, which will display individual relaxation behavior. Assuming superposed exponentials, an increase of the stretching factor would correspond to a narrower distribution of relaxation times (a Gaussian distribution would correspond to b = 0.67; Bakhmutov, 2012).

4.6 Conclusions

The applied dose rates, ranging from $5.31*10^{-4}$ to $7.45*10^{-3}$ Gy h^{-1}, influence the bacterial and the fungal community structure in the soil microcosms of the present study. We observed an inhibition as well as an enhancement of microbial species in the DGGE gel patterns. However, we did not detect any effects on the degradation of wheat straw or the chemical composition of soil organic matter and the findings in the ergosterol content could not be ascribed to the application of radionuclides. The sterilization/reinoculation procedure and the application of wheat straw had much stronger effects on the microbial community structure than the applied radionuclides. Recurring disturbances of the soil matrix by temperature and moisture changes, fertilization, pesticide treatment and mechanic impacts such as plowing will have an even more pronounced impact. As our microcosms were conducted under controlled laboratory conditions, extrapolation of our findings to the field scale will require further validation. Contaminations with ^{137}Cs or ^{90}Sr up to 50-fold that of the hotspots occurring in Chernobyl led to minor changes in soil microbial functions suggesting a strong resilience of natural soils to radioactive contamination.

5 Radiation induced impacts on the degradation of 2,4-D and the microbial population in soil microcosms[6]

5.1 Objectives

The objectives of this experiment were to investigate the effects of radioactive soil contamination on the microbial mineralization of 2,4-D. To obtain the effects on specialized 2,4-D degraders the 2,4-D was labeled with ^{14}C in the recalcitrant phenoxy ring. The mineralization rates were determined by analyzing the released $^{14}CO_2$. Changes in the microbial community structure were investigated with help of the DGGE.

5.2 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:

[6] Adapted from Niedrée, B., Vereecken, H., Burauel, P., 2013. Radiation induced impacts on the degradation of 2,4-D and the microbial population in soil microcosms. Journal of Environmental Radioactivity 115,168-174.

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 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: 0.1m). 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.

5.3 Material and methods

5.3.1 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 (≤ 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 hours.

5.3.2 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 (100µg mL^{-1}), the 2,4-D was applied to soil aliquots (5 g dry weight) which had previously been ashed at 600 °C for 24h. 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 minutes. 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).

Table 5.1: 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

5.3.3 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.

5.3.4 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 CCC GCG CCC GGC CCG CCG CCC CCG CCC CAT TCC CCG TTA CCC GTT G-3'). The denaturing gradient in the eubacterial 16S gel ranged from 5 to 40%, running time was 20 h at 60 °C with a current voltage of 50 V. The gel contained 6% polyacrylamide. In the 18S fungal gel, the denaturing gradient ranged from 5 to 40%, 100% denaturant corresponded to 7 M urea and 40% foramide. Running time was 20 h at 60 °C with a current voltage of 50 V. The gel contained 7% polyacrylamide. A detailed description of the method can be found in Niedrée et al. (2012). Due to limited space in the DGGE gels, from most of the soils only 2 or 3 amplification products were applied to the gel. Bands were visualized by silver staining. Gels were analysed with GelCompar II Version 3.0 (Applied Maths, Sint-Martens-Latem, Belgium). After normalizing the gels and substracting the background by the rolling ball method, the Pearson product-moment correlation was calculated. Pearson's correlation coefficient compares the densiometric curves of the fingerprint. The clustering of the patterns was calculated using the unweighted pair group method with arithmetic mean (UPGMA).

5.3.5 Statistical analyses

Mineralization data were analyzed using one-way analyses of variance (ANOVA). Statistical analyses were carried out using free software GNU R version 2.15.0.

5.4 Results

5.4.1 Mineralization of 2,4-D

Figures 5.1a to 5.1d show the percentage of evolved $^{14}CO_2$ (cumulative) of the uniformly ^{14}C-ring-labeled 2,4-dichlorophenoxyacetic acid in the microcosms treated with ^{137}Cs and ^{90}Sr, respectively. The native ^{137}Cs (Fig. 5.1a) and ^{90}Sr (Fig. 5.1c) treated soils show a similar course of mineralization. After a lag phase of at most 2 days mineralization in low (■) and medium (♦) contaminated soils as well as the control soils (▲) started and reached the plateau at day 10 accounting for 69 to 76% of the originally applied ^{14}C radioactivity. Up to day 30

the evolved $^{14}CO_2$ raised slightly to 79 to 84%. Just the high contaminated soils (Fig. 5.1a and 5.1c, ●) show a different mineralization pattern. The mineralization started after a lag phase of at most 6 (^{137}Cs) and 4 (^{90}Sr) days and reached the plateau after 16 days (^{137}Cs: 73%, ^{90}Sr: 76%) only rising up to 77% (^{137}Cs) and 80% (^{90}Sr) at day 30. From day 2 to 12 (Fig. 5.1a) the soils with the highest contamination of ^{137}Cs exhibit a mineralization significantly lower compared to other soils. In case of the ^{90}Sr treatment, the mineralization of the highly contaminated soils is lower for all days of incubation (Fig. 5.1c). The half-time of 2,4-D mineralization is 10 days for the most highly and 6 days for the lower contaminated soils.

The mineralization pattern of the sterilized and reinoculated soils (Fig. 5.1b and 5.1d) was comparable to the highly contaminated native soils. The lag phase in this case was 6 days; the plateau was reached after 18 days and accounted for 74 to 77% (Fig. 5.1b, ^{137}Cs) and 77 to 82% (Fig 5.1d, ^{90}Sr). The totally evolved ^{14}C after 30 days was between 81 and 82% (Fig. 5.1b, ^{137}Cs) and 82 and 86% (Fig 5.1d, ^{90}Sr). From day 2 to 6 (Fig. 5.1b and 5.1d) addressing the highly contaminated reinoculated soils the mineralization is significantly lower compared to the lower contaminated soils. 50% of the totally applied ^{14}C were emitted as $^{14}CO_2$ after 11 days in all reinoculated soil treatments.

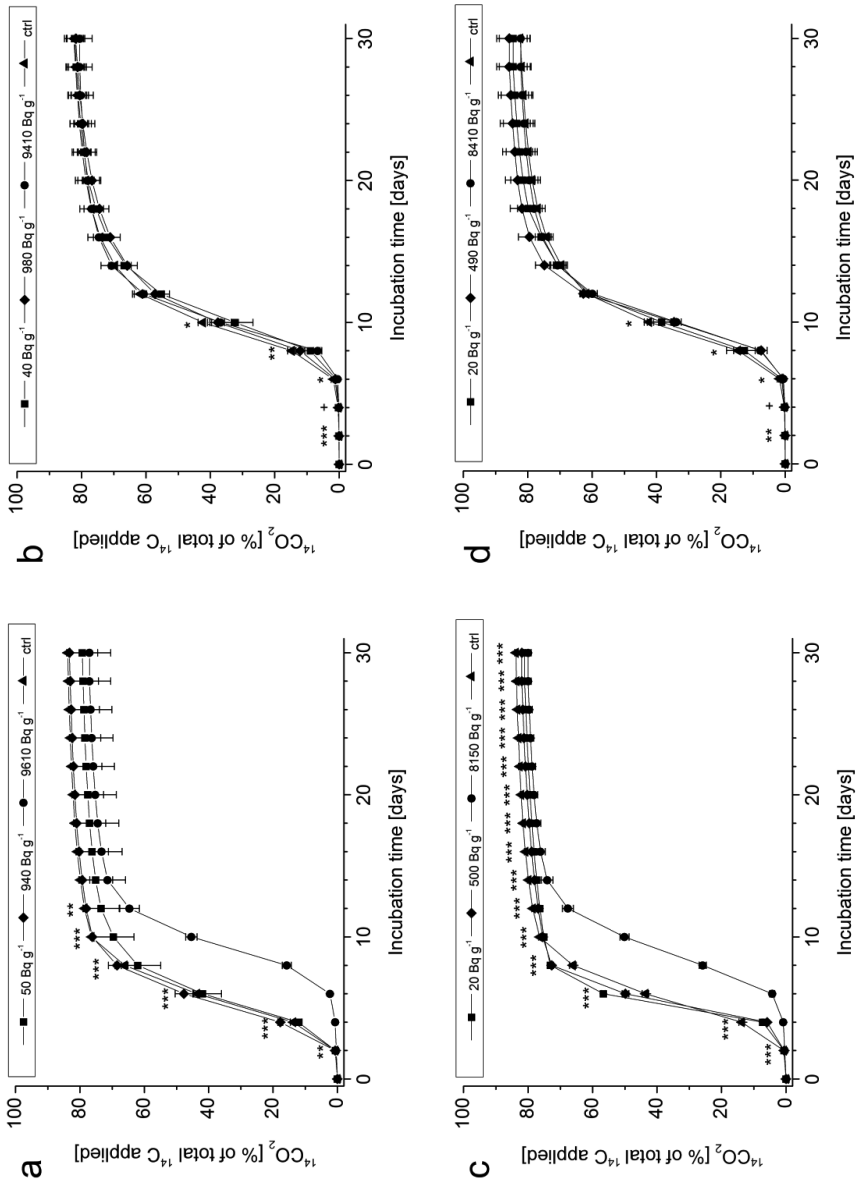


Figure 5.1: Cumulative $^{14}CO_2$ of ^{14}C-2,4-D mineralization (100 µg g^{-1}, uniformly-ring labeled) in native (a) and reinoculated (b) soils treated with ^{137}Cs and in native (c) and reinoculated (d) soils treated with ^{90}Sr. The vertical bars refer to the standard deviation of three replicates (applied radioactivity 100%). ANOVA significant levels: 0 ***, 0.001 **, 0.01*, 0.05^{+}.

5.4.2 Community composition

The development of the microbial community structure was compared using denaturing gradient gel electrophoresis. After 30 days, when the amounts of absorbed $^{14}CO_2$ remained stable and most of the 2,4-D was mineralized, incubation was stopped and the extracted DNA was amplified with primers for 16S and 18S rDNA. Bacterial band patterns can be seen in Figures 5.2a and 5.3a, fungal band patterns in Figures 5.2b and 5.3b. Cs1 and Sr1 represent the soils with lower contamination, Cs2 and Sr2 the medium contaminated and Cs3 and Sr3 the most highly contaminated soils. Ctrl are control soils to which neither ^{137}Cs nor ^{90}Sr were applied, but rather ^{14}C-labeled 2,4-D. Mzh represents pristine control soils from the field site Merzenhausen, these soils were neither incubated nor treated with ^{14}C-2,4-D or ^{137}Cs / ^{90}Sr.

The bacterial DGGE gel of the native soils (Fig. 5.2a) was characterized by numerous strong bands and numerous faint bands in between. The soils with medium contamination (Cs2 and Sr2) as well as ^{90}Sr soils with the lowest contamination (Sr1) were very similar. This was also represented in the cluster analysis. These soils formed a single group with a Pearson similarity of at least 65%. The next group was clustered by the most highly contaminated soils (Cs3 and Sr3) with a Pearson similarity of at least 56%. There is considerable disparity between them and the other, less contaminated soils in the lower part of the gel (circle 1). Here the bands are lacking which appear in the other lanes. The control soils (Ctrl) exhibited the greatest similarity to the soils with low ^{137}Cs contamination. Both treatments exhibited bands at position 2. The Pearson similarity in this latter cluster was at least 67%. The last group was formed by the completely untreated Merzenhausen soils. Here the Pearson similarity was at least 84%. However, the similarity to the closest group was less than 20%. Further remarkable disparities between the lanes were highlighted by positions 3 to 7.

In the bacterial band pattern produced from the reinoculated soils (Fig. 5.2b), the disparities were rather small. The Pearson similarity between all incubated soils was at least 69%. This cluster can be divided in two larger groups, soils with low and medium contamination (Pearson similarity at least 81%) and the most highly contaminated and the control soils (Pearson similarity at least 83%). The non-incubated soils (Mzh), as well as one lane of the most highly contaminated soils, formed a distinct group. The latter did not exhibit any visible bands.

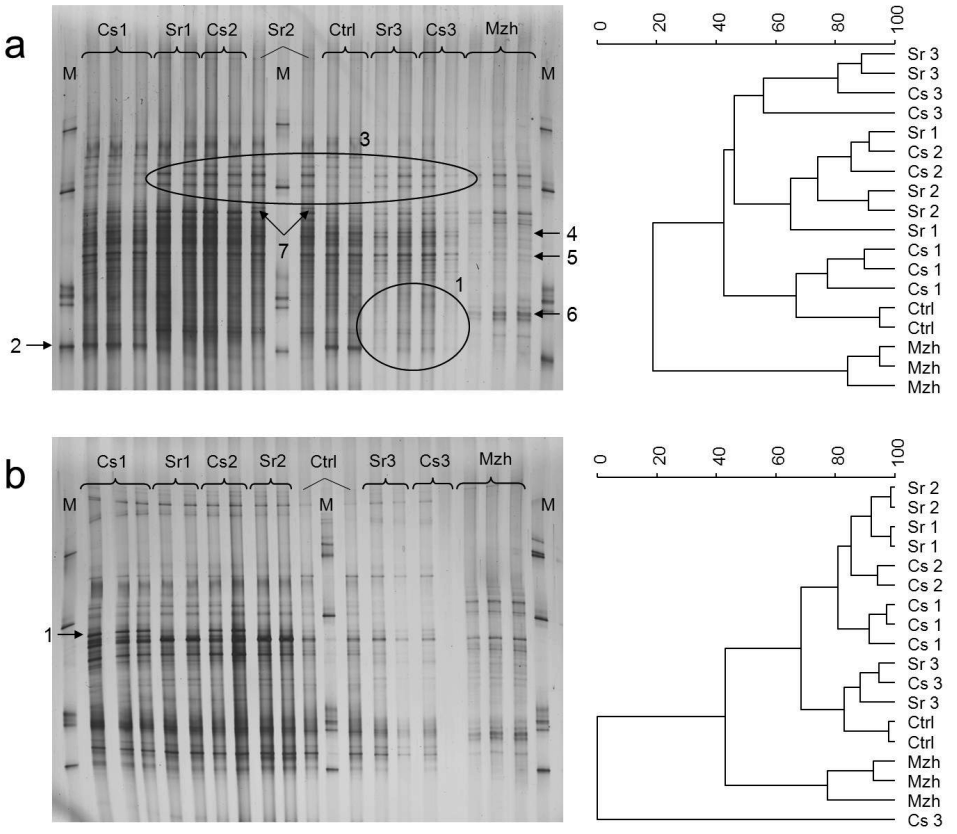


Figure 5.2: DGGE pattern and cluster analysis made from a) the 16S rDNA and b) the 18S rDNA of the native soil microcosms. Cs1 and Sr1 low, Cs2 and Sr2 medium and Cs3 and Sr3 high contamination. Mzh native soil from sampling site, not incubated, M markers. Denaturing gradient range 30 - 70%, acrylamide concentration 6%. Clustered with Pearson similarity correlation and UPGMA. Scale represents % similarity.

The 18S rDNA DGGE gels were characterized by a few strong gels with numerous faint bands in between (Fig. 5.3a). The lanes were clustered into four larger groups, whereas only a few disparities could be detected (Fig. 5.3a). One group was formed by low-, medium- and non-contaminated soils (Cs1, Sr1, Cs2, Sr2 and Mzh) with a Pearson similarity of at least 59%. The next group consisted of highly contaminated soils (Sr3 and Cs3) and non-contaminated control soils (Ctrl), respectively. The Pearson similarity here was at least 70%.

Two groups were formed by the most highly contaminated ^{137}Cs treated soils (Cs3) and soils with low contamination (Sr1 and Cs1), respectively. In the latter two soils, bands appeared at the position labelled with circle 1 which were absent in the other lanes. Remarkable disparities between control soils (Ctrl) and the radionuclide-treated soils are highlighted with arrows at position 2 and 3.

Similar to the native fungal gel, in the reinoculated fungal gel most of the bands were located in the upper part (Fig. 5.3b). In one of the strontium-treated soils with medium contamination (Sr2) bands according to bands in the marker lanes (M) appeared (circle). The similarity between the lanes was relatively high. However, slight disparities occured at the positions labeled with arrow 1 and arrow 2. The ^{137}Cs-treated soils with low contamination (Cs1) exhibited bands which were absent in the ^{90}Sr-treated soil (Sr1). The cluster analysis of the fungal band pattern of the reinoculated soils (Fig. 5.3b) was characterized by a heterogeneous lane distribution. Only a few lanes were congruent, such as Sr1 (97%), Mzh (81%), Sr3 (96%) and Cs3 (90%). The Pearson similarity is given in brackets. The other lanes were distributed and clustered irregularly. In one group, for example, control soils (Ctrl) and ^{90}Sr-treated soils with medium and high contamination (Sr2 and Sr3) were clustered with a Pearson similarity of at least 71%.

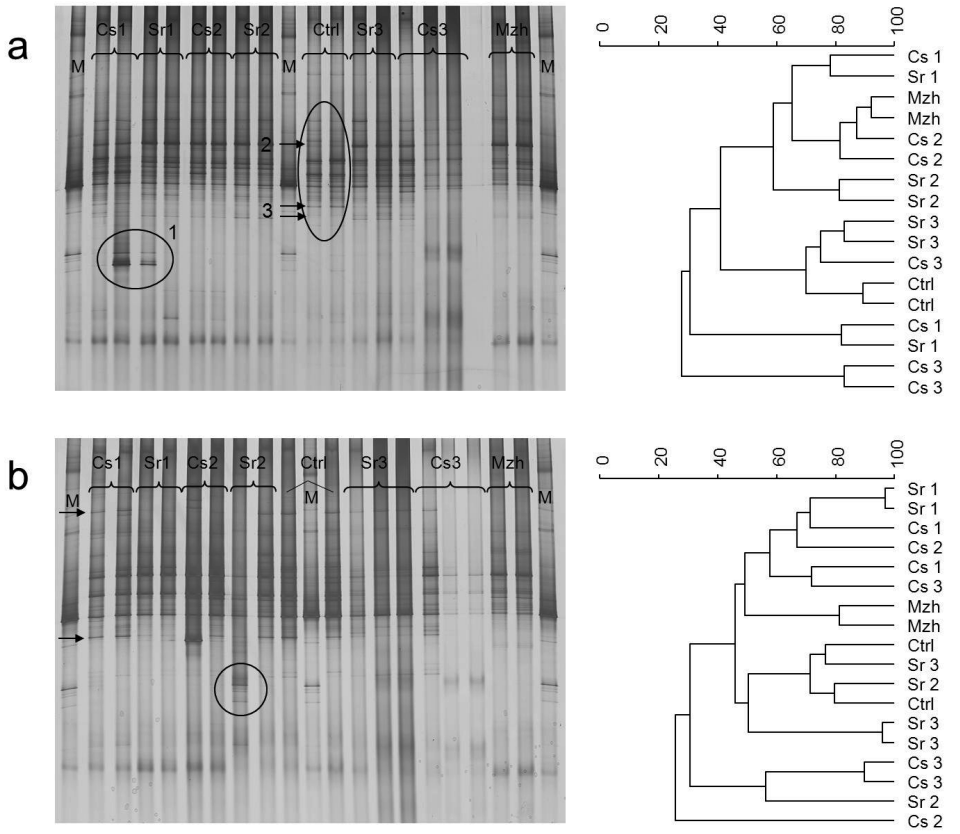


Figure 5.3: DGGE pattern and cluster analysis made from a) the 16S rDNA and b) the 18S rDNA of the sterilized and reinoculated soil microcosms. Cs1 and Sr1 low, Cs2 and Sr2 medium and Cs3 and Sr3 high contamination. Mzh native soil from sampling site, not incubated, M markers. Denaturing gradient range 30 - 70%, acrylamide concentration 6%. Clustered with Pearson similarity correlation and UPGMA. Scale represents % similarity.

5.5 Discussion

5.5.1 Mineralization of 2,4-D

The turnover of herbicides in soil depends, amongst other aspects, on the agricultural treatment of the soil. In recent years, the field was not treated with 2,4-D, but other herbicides were applied periodically, such as Carfentrazone-ethyl, Terbuthylazine, Triclopyr, Diuron,

Metazachlor, Quinmerac, Triasulfuron and Dicamba. The latter two are phenoxy herbicides, as is 2,4-D. Soil microorganisms are able to adapt to phenoxy herbicides, which results in an adaptation of the pristine microbial community and enhances and accelerates the degradation process (Loos et al., 1979; Smith and Aubin, 1994; Merini et al., 2007). This results in a short lag phase and fast turnover in native soils. Similar fast mineralization and short half-lives of 2,4-D are known from the literature; e.g. Shaw and Burns (1998): 50 - 60% of 20 µg g^{-1} 2,4-D, Stott et al. (1983): 66-74% of 25 µg g^{-1} 2,4-D or McCall (1981) 50% in an average of 4 days, 90% in an average of 11 days. The degradation of 2,4-D is performed typically by special 2,4-D degraders or by the co-metabolizing population (Soulas, 1993). In the latter case no lag phase is expected (Gonod et al., 2003). The occurrence of a lag phase in combination with high mineralization rates suggests that 2,4-D degraders were initially present, but reduced in number (Gonod et al., 2003).

Considering the reduction of the microbial community and a release of dissolved organic carbon (DOC) and total nitrogen (TN) due to sterilization and reinoculation (McNamara et al., 2003; Berns et al., 2008; Niedrée et al., 2012), the course of 2,4-D mineralization in the reinoculated soils can be assumed to be as follows. The supply of nutrients remobilized by autoclaving and the lower competition for space and nutrients supports the growth of the microbial community. Within the first days of incubation, the DOC and the acetic acid side chain of the 2,4-D became utilized and delivered carbon for the assimilation of microbial biomass (Forster and McKercher, 1973; Roberts et al., 1998). The microbial activity increased and after 6 days the microorganisms began to utilize the more recalcitrant radiolabeled dichlorphenyl ring (Tiedje and Alexander, 1969; Smith and Aubin, 1991; Roberts et al., 1998; Shaw and Burns, 1998; Young and Oh, 2006; Merini et al., 2007). Due to exclusive ^{14}C labeling of the phenyl ring, initially the mineralization of the acetic side chain did not result in a discharge of ^{14}C labeled carbon dioxide and visible mineralization did not begin until day 6. Moreover, the intensely growing microbial population incorporated some of the radiolabeled C or CO_2 (Santrucková et al., 2005; Feisthauer et al., 2008; Nowak et al., 2011), which could also reduce the release of $^{14}CO_2$. Gonod (2006) reported that during the lag phase the microbial community increased up to 12% of the initially applied radioactivity. With the beginning of mineralization and detectable release of $^{14}CO_2$, the population decreased again and stabilized at 5% of the initially applied radioactivity. Gonod applied 7.8 µg g^{-1} 2,4-D in aqueous solution. Forteen days after the first emissions of perceptible amounts of $^{14}CO_2$, the major proportion of the applied 2,4-D was mineralized in native and in reinoculated soils. The

further release of very low amounts of $^{14}CO_2$ was presumably caused by the mineralization of microbial storage polysaccharides and the mineralization of recalcitrant metabolites (Ward, 1985; Soulas, 1993).

Potential impacts caused by ionizing radiation could be seen in the mineralization of the native (Fig. 5.1a and 5.1c) and to a lesser extent, in the mineralization of the reinoculated soils (Fig. 5.1b and 5.1d) contaminated with high concentrations of ^{137}Cs and ^{90}Sr. The maximum mineralization and the lag phase were delayed for 2 to 4 days compared to less contaminated native soils. This was presumably caused by inhibition of the metabolic activity of 2,4-D degraders. We assume that after a few days, either the phenyl ring degraders adapted to the radiation or competing species, which also are able to degrade the dichlorophenyl ring, benefited from better substrate availability. In contrast to the sterilized soils, in native soils ^{137}Cs and ^{90}Sr were applied to the pristine microbial community. This certainly affected the development of the microbial community. In native soils, the microbial community was established and was consequently not able to adapt to changes of environmental parameters as fast as in sterilized soils.

5.5.2 Community composition

In contrast to the untreated and unincubated Merzenhausen lanes, in the lanes where 2,4-D was applied and in the incubated soil lanes more and stronger bands appear in native gels as well as in reinoculated lanes. This indicates that species benefited from the 2,4-D treatment and the incubation conditions (Figures 5.2 and 5.3). Assuming that the size of a functional group is related to its metabolic activity (Soulas, 1993), the recovery of the mineralization in the reinoculated soils (Fig. 5.1b and 5.1d) also supported these findings. A growth of microbial community after application of 2,4-D was also reported by Ou (1984), Cullimore (1981) and Robertson and Alexander (1994). Macur et al. (2007) concluded from their experiments that 2,4-D application may provide a temporary selective advantage for organisms which are able to utilize 2,4-D as a carbon source.

The radiation-induced impact on the 2,4-D mineralization over time was not reflected by visible shifts in the community structure. The DGGE method seemed to reach its limits. It only detects the most abundant species (Muyzer et al., 1993) and the bands do not necessarily represent the most functionally dominant microorganisms (Torsvik et al., 1990). Functional

shifts in the background population cannot be detected by the DGGE method (Kisand and Wikner, 2003). However, the bacterial shift in the native soils (Fig. 5.2a, arrow 1) is presumably forced by radiation impact. In the ^{137}Cs-treated lanes with the lowest contamination (Cs1) and in the control lanes (Ctrl), bands appear which are absent in the more highly contaminated soils (Fig. 5.2a, arrow 1). Indeed the radioactivity of the ^{90}Sr-treated soils was lower compared to the ^{137}Cs-treated soils (Cs1: 50 ± 1.9 Bq g^{-1}; Sr1: 20 ± 1.6 Bq g^{-1}). Nevertheless, due to the higher decay energy of ^{90}Sr (Keller, 1993) the dose rate affecting the microorganisms in ^{90}Sr-treated soils was also higher compared to the ^{137}Cs-treated soils (Table 5.1). The band distribution marked by circle 3 (Fig. 5.2a) also seemed to be radiation-induced. In the lanes with medium and highest contamination, bands were stronger compared to less or uncontaminated soils. A similar phenomenon was observed by McNamara et al. (2007). They assumed a competitive outgrowth by radiation-resistant bacteria. Indeed, they used external gamma radiation with much higher dose rates of up to 10 kGy.

The band distribution in the control soils (Ctrl) of the native fungal band pattern (Fig 5.3a) deviated from the other radionuclide-treated soils, even the least contaminated ones. Bands were absent or present in contrast to the other lanes (arrows 1 and 4). These findings suggest that even relatively low contaminations with ^{137}Cs and ^{90}Sr may affect the fungal community structure.

5.6 Conclusions and Outlook

It is not yet clarified whether the Chernobyl radionuclides ^{137}Cs and ^{90}Sr deposited in relatively low concentrations in Western Europe and in much higher concentrations in the Chernobyl exclusion zone influence the microbial community and its functions in soil. One important function is the decomposition of organic compounds, such as organic pesticides used in agriculture. In our study, we observed the mineralization of one representative herbicide, 2,4-dichlorophenoxyacetic acid, exposed to various doses of ionizing radiation. In addition to the mineralization of the ^{14}C-ring-labeled 2,4-D, the changes in microbial community were investigated using the bacterial and fungal rDNA DGGE. The uniformly ^{14}C-ring-labeled 2,4-D was found to be readily mineralized in all microcosms. In the soils with low contamination ranging up to 980 Bq g^{-1} (1.8*10^{-4} Gy h^{-1}) no effects on the mineralization rate could be seen. The highest soil contaminations of up to 9610 Bq g^{-1} (1.7*10^{-3} Gy h^{-1}) delayed 2,4-D mineralization in native soils temporarily. The lag phase was

extended for 4 days. The 2,4-D mineralization in the reinoculated soil was not affected. The bacterial and the fungal community shifted due to the radioactive contamination. However, the application of 2,4-D as well as the sterilization/reinoculation procedure affected the community structure much more strongly than the ionizing radiation. We assume that the ^{137}Cs and ^{90}Sr deposited in Belorussia, Ukraine and Russia and in Western Europe do not affect the mineralization of 2,4-D and possibly pesticides with a generally high mineralization potential.

6 Final remarks

6.1 General conclusions

During numerous accidents at nuclear power plants (NPP) and other nuclear facilities and due to nuclear bomb tests large amounts of various radionuclides were ejected into the environment. In case of damages to the fuel rods or even core melts, numerous artificial radionuclides appear. Most of these radionuclides have relatively short half-lifes, which means several weeks or years later only residues of these radionuclides can be found. However, their risk potential should not be neglected, as for example numerous people died by thyroid deseases caused by isotopes of iodine also blown out during the Chernobyl accident (Brenner et al., 2011). The half-life of ^{131}I is only 8 days. Other radionuclides have much longer half-lives. Two of them, which are typical for accidents at nuclear power plants are ^{137}Cs and ^{90}Sr. They were released in huge amounts from reactor 4 in Chernobyl in 1986 and distributed across Ukraine, Belarus, Russia and the European countries. The chemical and physical properties of both isotopes make them very interesting in various perspectives. Due to high temperatures caused by the burning moderator material (graphite) the ^{137}Cs rose into high altitudes, was transported with wind streams and was precipitated with the rain. Direct consequences to the human population were manifold, including relocation of the population and disposal of agricultural crops. Due to its half-life of 30.2 years and its high similarity to potassium, ^{137}Cs is still present in our environment. Upper mineral soil fixed much of the deposited cesium which accumulated in the fungi and subsequently in game, particularly wild boars (Fielitz, 2005; Konopleva et al., 2009). Thus, even almost 30 years after the Chernobyl accident, ^{137}Cs may end up in the human food chain. As it was mainly bound to larger particles, ^{90}Sr was not distributed across large distances (UNSCEAR, 2000). However, its half-life is about 28.8 years and even today strong contaminations around the reactor in Chernobyl can be measured (Kashparov et al., 2001).

Against the background of direct or indirect effects on humans, the radioecological research after the Chernobyl accident mainly focused on transport of radionuclides into plants, animals and the human food chain. Only a few studies considered radiation effects on ecological processes itself. The effects of radioactive contaminations on microorganisms and soils for example is almost unknown. For this reason a series of experiments were accomplished to obtain further knowledge on the impact of radionuclides on microbial communities and

microbial functions. All experiments were conducted at lab scale in soil microcosms. Soil, artificially contaminated with various concentrations of ^{137}Cs or ^{90}Sr (as nitrates) were applied with ^{14}C-labeled substrates and incubated under controlled laboratory conditions. The radionuclide treatment was intended to simulate the contamination appearing in the 10 km zone around Chernobyl. The lowest radionuclide treatment (~ 15 Bq g^{-1}) corresponded to the threshold for the exclusion zone, which starts at 12 Bq g^{-1}. However, applications in this range did not result in effects and thus were not considered further. The hotspots of ^{137}Cs contamination near Chernobyl exhibit values of about 320 Bq g^{-1}, which corresponded to ~ 500 Bq g^{-1} in the experiments. The highest applications in the experiments were about 15,000 Bq g^{-1}. For comparison: ^{137}Cs hotspots caused by the Chernobyl catastrophe in Germany are about 0.5 Bq g^{-1} . To obtain most significant results in the DGGE method, the microcosms were treated with native soil or with soil which was previously sterilized and reinoculated with a small aliquot of native soil. The process of the sterilization itself had a strong impact on the community structure and the microbial activities. The comparison of shifts in bacterial communitis was conducted with the 16S rDNA DGGE, while, in case of fungi the 18S rDNA DGGE was used.

In the first experiment the focus was on the impact of the radionuclides on the degradation of ^{14}C labeled wheat straw. 2% straw was applied to the microcosms and incubated for 70 days at 20 °C with a water content of 50% of the water-holding capacity (WHC). The radioactive contamination showed minor effects on the bacterial as well as the fungal community structure. In both bacterial and fungal DGGE gel patterns in contaminated soils bands disappeared, which is an evidence for negative effects on dominant microbial species. Although the community structure was affected, no impact on the mineralization of the wheat straw occurred. In agricultural practice wheat straw regularly remains on the field after the harvest or is used as a fertilizer. Thus the microbial community is adapted to this substrate and the mineralization starts immediately or without a lag phase. Presumably the lack of some negative effects is caused by a functional overlap. Hence, numerous different species participate in the degradation of the wheat straw and compete for the same nutrients, the inhibition of some species does not affect the turnover rates of the straw. Moreover, the DGGE method has a limited resolution. Only the most abundant microorganisms in number are represented in the gel patterns. The amount of the microorganisms bearing the investigated functions can be much smaller, so they possibly fade into the background.

In the second experiment the shift of the fungal biomass and the chemical composition of the soil organic matter under the influence of radionuclides was investigated. Native as well as sterilized and reinoculated microcosms were applied with wheat straw, various concentrations of ^{137}Cs or ^{90}Sr and were incubated for 3 months at 20 °C and a water content of 50% of the WHC. The fungal biomass was compared with help of the ergosterol content, extracted from soil. The formation of the humic substances was compared by using the solid state nuclear magnetic resonance spectroscopy (^{13}C CP/ MAS NMR). In both investigations no effects caused by the radiation could be observed. Conversely, in the community structure, shifts caused by the radioactive contamination occurred. In the fungal gel pattern these shifts were much more pronounced than in the bacterial gel. Interestingly, the radiation did not result only in inhibition, but also in an enhancement of fungal species. This effect is probably caused by indirect radiation effects: The radiation inhibits particular species, which results in a better nutrient supply and less competition for other species.

In the third experiment the effects of the applied radionuclides on the mineralization of the ^{14}C-labeled herbicide 2,4-D were observed. The experimental setup was similar to the first experiment conducted with radio-labeled wheat straw. The mineralization of the 2,4-D was inhibited for 4 days, which could be ascribed to the contamination with ^{137}Cs or ^{90}Sr, respectively. These effects only occurred in the native soils with the highest concentration from 8150 to 9610 Bq g^{-1}, which is approximately 30-fold that of the maximum contamination near Chernobyl. The 2,4-D was uniformly labeled with ^{14}C in the phenylic ring structure, which is more recalcitrant than the acetic side chain. It is assumed that only a small group of specialized microorganisms is able to degrade this ring structure. Thus a functional overlap can be excluded. However, after a few days the mineralization of the 2,4-D recovers and the mineralization rates align with the control soils without radioactive contamination. As in the other experiments here also slight shifts in the microbial community structure were observed which could be related to the applied radioactivity.

Considering the fact that agricultural treatment is prohibited in the Chernobyl exclusion zone, which is relatively low contaminated compared to our microcosms, it is suggested that microbial functions are not affected by accidently released ^{137}Cs and ^{90}Sr in a realistic order of magnitude.

6.2 Outlook

It can be concluded for all experiments that ^{137}Cs and ^{90}Sr affect the bacterial and the fungal community structure. Several species were reduced in their amount, others were enhanced. Microbial functions were not affected, or only affected temporarily. ^{137}Cs and ^{90}Sr concentrations in the experiments accounted for at maximum 30 to 50-fold that of the highest hotspots occurring near Chernobyl and more than 1300 more than the threshold value for the exclusion zone. Compared to the ^{137}Cs concentrations which can be found in Western Europe (e.g. Sweden, 184 kBq m^{-2}), the maximum applied concentration exceed the factor 9700.

All experiments were conducted under controlled conditions in the laboratory; the soil water content as well as the temperature were known and kept constant and except for soil sampling in the NMR experiment and changing of the NaOH traps, the microcosms were not disturbed during incubation. For agricultural soils under realistic conditions this is different, as these soils are disturbed recurringly by plowing, fertilizing, pest treatment, crop growing and changing weather conditions. Regular plowing for example leads to a further dilution of the radionuclides and fertilizing and remaining crop residues promote the growth of the microbial biomass exceedingly. Thus it is expected, that the slight effects visible in our laboratory experiments are diminished or overlaid by other biotic and abiotic effects under real field conditions.

7 References

Alexakhin, R.M., Sanzharova, N.I., Fesenko, S.V., Spiridonov, S.I., Panov, A.V., 2007. Chernobyl radionuclide distribution, migration, and environmental and agricultural impacts. Health Phys. 93, 418-426.

Amann, R.I., Ludwig, W., Schleifer, K.-H., 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. 59, 143 - 169.

Andren, O., Bengtsson, J., Clarholm, M., 1995. Biodiversity and Species Redundancy among Litter Decomposers. Kluwer Acedemic Publishers.

Aneja, M.K., Sharma, S., Fleischmann, F., Stich, S., Heller, W., Bahnweg, G., Munch, J.C., Schloter, M., 2006. Microbial colonization of beech and spruce litter - Influence of decomposition site and plant litter species on the diversity of microbial community. J. Microbiol. Methods 52, 127-135.

Auerbach, S.I., 1986. Comparative forest ecosystem behavior of three long-lived radionuclides. in: Communities, C.o.E. (Ed.). Proceedings on the Cycling of Long-Lived Radionuclides in the Biosphere: Observations and Models, Madrid, Spain.

Bakhmutov, V.I., 2012. Solid-State NMR in Materials Science, Principles and Applications. CRC Press, Boca Raton.

Bakken, L.R., Olsen, R.A., 1990. Accumulation of Radiocesium in Fungi. Can. J. Microbiol. 36, 704-710.

Bardgett, R.D., Jones, A.C., Jones, D.L., Kemmitt, S.J., Cook, R., Hobbs, P.J., 2001. Soil microbial community patterns related to the history and intensity of grazing in sub-montane ecosystems. Soil Biol. Biochem. 33, 1653-1664.

Bauer, J., Kirschbaum, M.U.F., Weihermuller, L., Huisman, J.A., Herbst, M., Vereecken, H., 2008. Temperature response of wheat decomposition is more complex than the common approaches of most multi-pool models. Soil Biol. Biochem. 40, 2780-2786.

Bermingham, S., Maltby, L., Cooke, R.C., 1995. A critical assessment of the validity of ergosterol as an indicator of fungal biomass. Mycol. Res. 99, 479-484.

Berns, A.E., Conte, P., 2010. Effect of RF field inhomogeneity and sample restriction on spectral resolution of CP/MAS-13C NMR spectra of natural organic matter. Open Magn. Res. J. 3, 75-83.

Berns, A.E., Philipp, H., Narres, H.D., Burauel, P., Vereecken, H., Tappe, W., 2008. Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. Eur. J. Soil Sci. 59, 540-550.

Berreck, M., Haselwandter, K., 2001. Effect of the arbuscular mycorrhizal symbiosis upon uptake of cesium and other cations by plants. Mycorrhiza 10, 275 - 280.

Bilo, M., Steffens, W., Fuhr, F., Pfeffer, K.H., 1993. Uptake of $^{134/137}$Cs in soil by cereals as a function of several soil parameters of three soil types in Upper Swabia and North Rhine-Westphalia (FRG). J. Environ. Radioact. 19, 25-39.

Blagodatskaya, E., Yuyukina, T., Blagodatsky, S., Kuzyakov, Y., 2010. Turnover of soil organic matter and of microbial biomass under C-3-C-4 vegetation change: Consideration of C-13 fractionation and preferential substrate utilization. Soil Biol. Biochem. 43, 159 - 166.

Blanco, M.J., Almendros, G., 1997. Chemical transformation, phytotoxicity and nutrient availability in progressive composting stages of wheat straw. Plant Soil 196, 15-25.

Borovoi, A.A., Gagarinskii, A.Y., 2001. Emission of radionuclides from the destroyed unit of the Chernobyl nuclear power plant. At. Energy 90, 153-161.

Bossew, P., Ditto, M., Falkner, T., Henrich, E., Kienzl, K., Rappelsberger, U., 1996. Cäsiumbelastung der Böden Österreichs. Monographien Band 60. Umweltbundesamt, Vienna, Austria.

Brenner, A.V., Tronko, M.D., Hatch, M., Bogdanova, T.I., Oliynik, V.A., Lubin, J.H., Zablotska, L.B., Tereschenko, V.P., McConnell, R.J., Zamotaeva, G.A., O'Kane, P., Bouville, A.C., Chaykovskaya, L.V., Greenebaum, E., Paster, I.P., Shpak, V.M., Ron, E., 2011. I-131 Dose Response for Incident Thyroid Cancers in Ukraine Related to the Chornobyl Accident. Environ. Health Persp. 119, 933-939.

Brueckmann, A., Wolters, V., 1994. Microbial immobilization and recycling of ^{137}Cs in the organic layers of forest ecosystems: Relationship to environmental conditions, humification and invertebrate activity. Sci. Total Environ. 157, 249-256.

Bundesministerium für Ernährung, L.u.V.H., 2010. Statistisches Jahrbuch über Ernährung, Landwirtschaft und Forsten der Bundesrepublik Deutschland Wiesbaden.

Burauel, P., Baßmann, F., 2005. Soils as filter and buffer for pesticides - experimental concepts to understand soil functions. Environ Pollut 133, 11-16.

Calmon, P., Thiry, Y., Zibold, G., Rantavaara, A., Fesenko, S., 2009. Transfer parameter values in temperate forest ecosystems: a review. J. Environ. Radioact. 100, 1-10.

Chapin, F.S., 1997. Biotic control over the functioning of ecosystems. Science 277, 500-504.

Clint, G.M., Dighton, J., 1992. Uptake and accumulation of radiocaesium by mycorrhizal and non-mycorrhizal heather plants. New Phytol. 121, 555-561.

Coughtrey, P.J., Thorne, M.C., 1983. Radionuclide Distribution and Transport in Terrestrial and Aquatic Ecosystems. Taylor & Francis, Rotterdam.

Cullimore, D.R., 1981. The enumeration of 2,4-D degraders in Saskatchewan soils. Weed Sci. 29, 440 - 443.

Dadachova, E., Bryan, R.A., Huang, X., Moadel, T., Schweitzer, A.D., Aisen, P., Nosanchuk, J.D., Casadevall, A., 2007. Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. PLoS One 2, Article.

Daly, M.J., 2009. A new perspective on radiation resistance based on Deinococcus radiodurans. Nat. Rev. Microbiol. 7, 237-245.

Davis, R.J., Sheldon, V.L., Auerbach, S.I., 1956. Lethal effects of gamma radiation upon segments of a natural microbial population. J. Bacteriol. 72, 505-510.

Declerck, S., Dupre de Boulois, H., Bivort, C., Delvaux, B., 2003. Extraradical mycelium of the arbuscular mycorrhizal fungus Glomus lamellosum can take up, accumulate and translocate radiocaesium under root-organ culture conditions. Environ. Microbiol. 5, 510-516.

Dederichs, H., Pillath, J., Heuel-Fabianek, B., Hill, P., Lennartz, R., 2009. Langzeitbeobachtung der Dosisbelastung der Bevölkerung in radioaktiv kontaminierten Gebieten Weissrusslands :Korma-Studie. in: GmbH, F.J. (Ed.). Energy and Environment. Forschungszentrum Jülich GmbH, Jülich.

Delvaux, B., Agapkina, G.I., Bunzl, K., Rafferty, B., Kliashtorin, A., Kruyts, N., Maes, E., 1996. Mechanisms controlling radionuclide mobility in forest soils. The Radiological Consequences of the Chernobyl Accident, Brussels, Luxembourg, pp. 193-196.

Dighton, J., Clint, G.M., Poskitt, J., 1991. Uptake and accumulation of Cs-137 by upland grassland soil fungi - a potential pool of Cs immobilization. Mycol. Res. 95, 1052-1056.

Dilly, O., Bloem, J., Vos, A., Munch, J.C., 2004. Bacterial diversity in agricultural soils during litter decomposition. Appl. Environ. Microbiol. 70, 468-474.

Drissner, J., Burmann, W., Enslin, F., Heider, R., Klemt, E., Miller, R., Schick, G., Zibold, G., 1998. Availability of caesium radionuclides to plants - Classification of soils and role of mycorrhiza. J. Environ. Radioact. 41, 19-32.

Dumat, C., Staunton, S., 1999. Reduced adsorption of caesium on clay minerals caused by various humic substances. J. Environ. Radioact. 46, 187-200.

Dupré De Boulois, H., Delvaux, B., Declerck, S., 2005. Effects of arbuscular mycorrhizal fungi on the root uptake and translocation of radiocaesium. Environ. Pollut. 134, 515-524.

Dupré de Boulois, H., Joner, E.J., Leyval, C., Jakobsen, I., Chen, B.D., Roos, P., Thiry, Y., Rufyikiri, G., Delvaux, B., Declerck, S., 2008. Role and influence of mycorrhizal fungi on radiocesium accumulation by plants. J. Environ. Radioact. 99, 785-800.

Eash, N.S., Stahl, P.D., Parkin, 1996. A simplified method for extraction of ergosterol from soil. Soil Science Society of America Journal 60, 468-471.

Ekschmitt, K., Klein, A., Pieper, B., Wolters, V., 2001. Biodiversity and functioning of ecological communities - Why is diversity important in some cases and unimportant in others? J. Plant Nutr. Soil Sci. 164, 239-246.

El-Sayed, W., Ghanem, S., 2009. Bacterial community structure change induced by gamma irradiation in hydrocarbon contaminated and uncontaminated soils revealed by PCR-denaturing gradient gel electrophoresis. Biotechnol. J. 8, 78-85.

Eriksen, W.H., Emborg, C., 1978. Increase of radiation resistance of a soil microflora exposed to long-term gamma-irradiation. Appl. Environ. Microbiol. 36, 618-619.

Feisthauer, S., Wick, L.Y., Kastner, M., Kaschabek, S.R., Schlomann, M., Richnow, H.H., 2008. Differences of heterotrophic $^{13}CO_2$ assimilation by Pseudomonas knackmussii strain B13 and Rhodococcus opacus 1CP and potential impact on biomarker stable isotope probing. Environ. Microbiol. 10, 1641-1651.

Ferris, M.J., Nold, S.C., Revsbech, N.P., Ward, D.M., 1997. Population structure and physiological changes within a hot spring microbial mat community following disturbance. Appl. Environ. Microbiol. 63, 1367-1374.

Fielitz, U., 2005. Untersuchungen zum Verhalten von Radiocäsium in Wildschweinen und anderen Biomedien des Waldes. Schriftenreihe Reaktorsicherheit und Strahlenschutz, BMU-2005-675. Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit.

Forster, R.K., McKercher, R.B., 1973. Laboratory incubation studies of chlorophenoxyacetic acids in chernozemic soils. Soil Biol. Biochem. 5, 333-337.

Franz, E.H., Woodwell, G.M., 1973. Effects of chronic gamma-irradiation on soil algal community of an oak-pine forest. Radiat. Bot. 13, 323-329.

Fredrickson, J.K., Zachara, J.M., Balkwill, D.L., Kennedy, D., Li, S.-m.W., Kostandarithes, H.M., Daly, M.J., Romine, M.F., Brockman, F.J., 2004. Geomicrobiology of high-level nuclear waste-contaminated vadose sediments at the Hanford site, Washington State. Appl. Environ. Microbiol. 70, 4230-4241.

Fukuyama, T., Takenaka, C., 2004. Upward mobilization of ^{137}Cs in surface soils of Chamaecyparis obtusa Sieb. et Zucc. (hinoki) plantation in Japan. Sci. Total Environ. 318, 187-195.

Fuma, S., Ishii, N., Takeda, H., Miyamoto, K., Yanagisawa, K., Ichimasa, Y., Saito, M., Kawabata, Z., Polikarpov, G.G., 2003. Ecological effects of various toxic agents on the aquatic microcosm in comparison with acute ionizing radiation. J. Environ. Radioact. 67, 1-14.

Fuma, S., Takeda, H., Miyamoto, K., Yanagisawa, K., Inoue, Y., Sato, N., Hirano, M., Kawabata, Z., 1998. Effects of gamma-rays on the populations of the steady-state ecological microcosm. Int. J. Radiat. Biol. 74, 145-150.

Gadd, G.M., 1996. Influence of microorganisms on the environmental fate of radionuclides. Endeavour 20, 150-156.

Garg, N., Chandel, S., 2010. Arbuscular mycorrhizal networks: process and functions. A review. Agron. Sustainable Dev. 30, 581-599.

Gastberger, M., Steinhausler, F., Gerzabek, M.H., Lettner, H., Hubmer, A., 2000. Soil-to-plant transfer of fallout caesium and strontium in Austrian lowland and Alpine pastures. J. Environ. Radioact. 49, 217-233.

Gessner, M.O., Schwoerbel, J., 1991. Fungal biomass associated with decaying leaf litter in a stream. Oecologia 87, 602-603.

Gochenaur, S.E., Woodwell, G.M., 1974. Soil microfungi of a chronically irradiated oak-pine forest. Ecology 55, 1004-1016.

Gonod, L.V., Chenu, C., Soulas, G., 2003. Spatial variability of 2,4-dichlorophenoxyacetic acid (2,4-D) mineralisation potential at a millimetre scale in soil. Soil Biol. Biochem. 35, 373-382.

Gonod, L.V., Martin-Laurent, F., Chenu, C., 2006. 2,4-D impact on bacterial communities, and the activity and genetic potential of 2,4-D degrading communities in soil. FEMS Microbiol. Ecol. 58, 529-537.

Griffiths, B.S., Kuan, H.L., Ritz, K., Glover, L.A., McCaig, A.E., Fenwick, C., 2004. The relationship between microbial community structure and functional stability, tested experimentally in an upland pasture soil. Microb. Ecol. 47, 104-113.

Griffiths, B.S., Ritz, K., Bardgett, R.D., Cook, R., Christensen, S., Ekelund, F., Sorensen, S.J., Baath, E., Bloem, J., de Ruiter, P.C., Dolfing, J., Nicolardot, B., 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity-ecosystem function relationship. Oikos 90, 279-294.

Griffiths, E., Burns, R.G., 1968. Effects of gamma irradiation on soil aggregate stability. Plant Soil 28, 169–172.

Guillitte, O., Melin, J., Wallberg, L., 1994. Biological pathways of radionuclides originating from the Chernobyl fallout in a boreal forest ecosystem. Sci. Total Environ. 157, 207-215.

Haas, R., 2001. Vertikale und laterale Verteilung von Cäsium-137 an Hängen unter Dauergrünland in Luxemburg, Trier.

Holland, E.A., Coleman, D.C., 1987. Litter placement effects on microbial and organic matter dynamics in an agroecosystem. Ecology 68, 425-433.

Ivanov, Y.A., Lewyckyj, N., Levchuk, S.E., Prister, B.S., Firsakova, S.K., Arkhipov, N.P., Arkhipov, A.N., Kruglov, S.V., Alexakhin, R.M., Sandalls, J., Askbrant, S., 1997. Migration of /sup 137/Cs and /sup 90/Sr from Chernobyl fallout in Ukrainian, Belarussian and Russian soils. J. Environ. Radioact. 35, 1-21.

Joner, E.J., Roos, P., Jansa, J., Frossard, E., Leyval, C., Jakobsen, I., 2004. No Significant Contribution of Arbuscular Mycorrhizal Fungi to Transfer of Radiocesium from Soil to Plants. Appl. Environ. Microbiol. 70, 6512-6517.

Jones, H.E., West, H.M., Chamberlain, P.M., Parekh, N.R., Beresford, N.A., Crout, N.M.J., 2004. Effects of gamma irradiation on Holcus lanatus (Yorkshire fog grass) and associated soil microorganisms. J. Environ. Radioact. 74, 57-71.

Kashparov, V.A., Ahamdach, N., Zvarich, S.I., Yoschenko, V.I., Maloshtan, I.M., Dewiere, L., 2004. Kinetics of dissolution of Chernobyl fuel particles in soil in natural conditions. J. Environ. Radioact. 72, 335-353.

Kashparov, V.A., Lundin, S.M., Khomutinin, Y.V., Kaminsky, S.P., Levchuk, S.E., Protsak, V.P., Kadygrib, A.M., Zvarich, S.I., Yoschenko, V.I., Tschiersch, J., 2001. Soil contamination with ^{90}Sr in the near zone of the Chernobyl accident. J. Environ. Radioact. 56, 285-298.

Kashparov, V.A., Lundin, S.M., Zvarych, S.I., Yoshchenko, V.I., Levchuk, S.E., Khomutinin, Y.V., Maloshtan, I.M., Protsak, V.P., 2003. Territory contamination with the radionuclides representing the fuel component of Chernobyl fallout. Sci. Total Environ. 317, 105-119.

Kasteel, R., Burkhardt, M., Giesa, S., Vereecken, H., 2005. Characterization of field tracer transport using high-resolution images. Vadose Zone J. 4, 101-111.

Keller, C., 1993. Grundlagen der Radiochemie. Otto Salle Verlag und Verlag Sauerländer, Frankfurt.

Kimura, S., Ishidou, E., Kurita, S., Suzuki, Y., Shibato, J., Rakwal, R., Iwahashi, H., 2006. DNA microarray analyses reveal a post-irradiation differential time-dependent gene expression profile in yeast cells exposed to X-rays and gamma-rays. Biochem. Biophys. Res. Commun. 346, 51-60.

Kisand, V., Wikner, J., 2003. Limited resolution of 16S rDNA DGGE caused by melting properties and closely related DNA sequences. J. Microbiol. Meth. 54, 183-191.

Knicker, H., Luedemann, H.D., 1995. N-15 and C-13 CPMAS and solution NMR studies of N-15 enriched plant material during 600 days of microbial degradation. Org. Geochem. 23, 329-341.

Kohlmeier, S., Smits, T.H.M., Ford, R.M., Keel, C., Harms, H., Wick, L.Y., 2005. Taking the fungal highway: Mobilization of pollutant-degrading bacteria by fungi. Environ. Sci. Technol. 39, 4640-4646.

Konoplev, A.V., Viktorova, N.V., Virchenko, E.P., Popov, V.E., Bulgakov, A.A., Desmet, G.M., 1993. Influence of agricultural countermeasures on the ratio of different chemical forms of radionuclides in soil and soil solution. Sci. Total Environ. 137, 147-162.

Konopleva, I., Klemt, E., Konoplev, A., Zibold, G., 2009. Migration and bioavailability of ^{137}Cs in forest soil of southern Germany. J. Environ. Radioact. 100, 315-321.

Korneev, N.A., Povalyaev, A.P., Aleksakhin, R.M., Panteleev, L.I., Ratnikov, A.N., Kruglov, S.V., Sanzharova, N.I., Isamov, N.N., Sirotkin, A.N., 1988. Agroindustrial production sphere-radiological consequences of the Chernobyl accident and the chief protective measures. At. Energy 65, 677-683.

Korystov, Y.N., 1992. Contributions of the direct and indirect effects of ionizing radiation to reproductive cell death. Radiat. Res. 129, 228-234.

Kruyts, N., Delvaux, B., 2002. Soil organic horizons as a major source for radiocesium biorecycling in forest ecosystems. J. Environ. Radioact. 58, 175-190.

Ladeyn, I., Plassard, C., Staunton, S., 2008. Mycorrhizal association of maritime pine, Pinus pinaster, with Rhizopogon roseolus has contrasting effects on the uptake from soil and root-to-shoot transfer of Cs-137, Sr-85 and Tc-95m. J. Environ. Radioact. 99, 853-863.

Lensi, R., Lescure, C., Steinberg, C., Savoie, J.M., Faurie, G., 1991. Dynamics of residual enzyme activities denitrification potential and physico-chemical properties in a gamma-sterilized soil. Soil Biol. Biochem. 23, 367-374.

Lerch, T.Z., Dignac, M.F., Barriuso, E., Bardoux, G., Mariotti, A., 2007. Tracing 2,4-D metabolism in Cupriavidus necator JMP134 with C-13-labelling technique and fatty acid profiling. J. Microbiol. Meth. 71, 162-174.

Liebich, J., Schaffer, A., Burauel, P., 2003. Structural and functional approach to studying pesticide side-effects on specific soil functions. Environ. Toxicol. Chem. 22, 784-790.

Liebich, J., Vereecken, H., Burauel, P., 2006. Microbial community changes during humification of ^{14}C-labelled maize straw in heat-treated and native Orthic luvisol. Eur. J. Soil Sci. 57, 446 - 455.

Lieser, K.H., Steinkopff, T., 1989. Chemistry of radioactive cesium in the hydrosphere and in the geosphere. Radiochim. Acta 46, 39-47.

Loos, M.A., Schlosser, I.F., Mapham, W.R., 1979. Phenoxy herbicide degradation in soils: Quantitative studies of 2,4-D- and MCPA-degrading microbial populations. Soil Biol. Biochem. 11, 377-385.

Macur, R.E., Wheeler, J.T., Burr, M.D., Inskeep, W.P., 2007. Impacts of 2,4-D application on soil microbial community structure and on populations associated with 2,4-D degradation. Microbiol. Res. 162, 37-45.

Mandelbaum, R.T., Allan, D.L., Wackett, L.P., 1995. Isolation and characterization of a Pseudomonas sp that mineralizes the S-Triazine herbicide Atrazine. Appl. Environ. Microbiol. 61, 1451-1457.

Marschner, B., Bredow, A., 2002. Temperature effects on release and ecologically relevant properties of dissolved organic carbon in sterilised and biologically active soil samples. Soil Biol. Biochem. 34, 459-466.

Marschner, P., Umar, S., Baumann, K., 2010. The microbial community composition changes rapidly in the early stages of decomposition of wheat residue. Soil Biol. Biochem. 43, 445-451.

May, L.A., Smiley, B., Schmidt, M.G., 2001. Comparative denaturing gradient gel electrophoresis analysis of fungal communities associated with whole plant corn silage. Can. J. Microbiol. 47, 829-841.

McCall, P.J., Vrona, S.A., Kelley, S.S., 1981. Fate of Uniformly Carbon- 14 Ring Labeled 2,4,5-Trichlorophenoxyacetic Acid and 2,4-Dichlorophenoxyacetic Acid. J. Agric. Food Chem. 29, 100-107.

McNamara, N.P., Black, H.I.J., Beresford, N.A., Parekh, N.R., 2003. Effects of acute gamma irradiation on chemical, physical and biological properties of soils. Appl. Soil Ecol. 24, 117-132.

McNamara, N.P., Griffiths, R.I., Tabouret, A., Beresford, N.A., Bailey, M.J., Whiteley, A.S., 2007. The sensitivity of a forest soil microbial community to acute gamma-irradiation. Appl. Soil Ecol. 37, 1-9.

Merini, L.J., Cuadrado, V., Flocco, C.G., Giulietti, A.M., 2007. Dissipation of 2,4-D in soils of the Humid Pampa region, Argentina: a microcosm study. Chemosphere 68, 259-265.

Monib, M., Salam, M.A.A., Elhadidy, T.T., 1971. Cumulative effect of gamma irradiation on microbial soil population and its activity. Folia Microbiol. 16, 457-464.

Morris, S.J., Blackwood, C.B., 2007. The ecology of soil organisms. in: Paul, E.A. (Ed.). Soil Microbiology, Ecology, and Biochemistry. Academic Press, Burlington, pp. 195-229.

Mueller-Lemans, H., Van Dorp, F., 1996. Bioturbation as a mechanism for radionuclide transport in soil: Relevance of earthworms. J. Environ. Radioact. 31, 7-20.

Muyzer, G., De Waal, E.D., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59, 695-700.

Nicastro, A.J., Vreeland, R.H., Rosenzweig, W.D., 2002. Limits imposed by ionizing radiation on the long-term survival of trapped bacterial spores: beta radiation. Int. J. Radiat. Biol. 78, 891-901.

Niedrée, B., Vereecken, H., Burauel, P., 2012. Effects of low-level radioactive soil contamination and sterilization on the degradation of radiolabeled wheat straw. J. Environ. Radioact. 109, 29-35.

Nisbet, A.F., Woodman, R.F.M., 2000. Soil-to-plant transfer factors for radiocesium and radiostrontium in agricultural systems. Health Phys. 78, 279-288.

Nowak, K.M., Mltner, A., Gehre, M., Schaffer, A., Kastner, M., 2011. Formation and fate of bound residues from microbial biomass during 2,4-D degradation in soil. Environ. Sci. Technol. 45, 999-1006.

OECD, 2002. Organisation for Economic Co-operation and Development (OECD), Guideline for Testing of Chemicals, No. 307. Aerobic and anaerobic transformation in soil, Paris.

Ou, L.-T., 1984. 2,4-D degradation and 2,4,-D degrading microorganisms in soils. J. Soil Sci. 137, 100-107.

Parekh, N.R., Poskitt, J.M., Dodd, B.A., Potter, E.D., Sanchez, A., 2008. Soil microorganisms determine the sorption of radionuclides within organic soil systems. J. Environ. Radioact. 99, 841-852.

Pitonzo, B.J., Amy, P.S., Rudin, M., 1999a. Effect of gamma radiation on native endolithic microorganisms from a radioactive waste deposit site. Radiat. Res. 152, 64-70.

Pitonzo, B.J., Amy, P.S., Rudin, M., 1999b. Resuscitation of microorganisms after gamma irradiation. Radiat. Res. 152, 71-75.

Poinsot-Balaguer, N., Castet, R., Tabone, E., 1991. Impact of chronic gamma irradiation on a Mediterranean forest ecosystem in Cadarache (France). J. Environ. Radioact. 14, 23-36.

Pyuskyulyan, K., 2006. The net effect of the Armenian nuclear power plant on the environment and population compared to the background from global radioactive fallout. in: Atoyan, V., Apakelyan, V., Saghatelyan, A. (Eds.). NATO Advanced Research Workshop of Radiological Risks in Central Asia. Springer Netherlands, Almaty, Kazakhstan, pp. 125-131.

Rafferty, B., Brennan, M., Dawson, D., Dowding, D., 2000. Mechanisms of Cs-137 migration in coniferous forest soils. J. Environ. Radioact. 48, 131-143.

Rafferty, B., Dawson, D., Kliashtorin, A., 1997. Decomposition in two pine forests: the mobilisation of ^{137}Cs and K from forest litter. Soil Biol. Biochem. 29, 1673-1681.

Riesen, T.K., Brunner, I., 1996. Effect of ectomycorrhizae and ammonium on ^{134}Cs and ^{85}Sr uptake into Picea abies seedlings. Environ. Pollut. 93, 1-8.

Roberts, T.R., Hutson, D.H., Lee, P.W., Nicholls, P.H., Plimmer, J.R. (Eds.), 1998. Metabolic Pathways of Agrochemicals: Part 1. The Royal Society of Chemistry, Cambridge, UK.

Robertson, B.K., Alexander, M., 1994. Growth-linked and cometabolic biodegradation: possible reason for occurrence or absence of accelerated pesticide biodegradation. Pestic. Sci. 41, 311 - 318.

Rocha, W.S.D., Regitano, J.B., Alleoni, L.R.F., 2006. 2,4-D residues in aggregates of tropical soils as a function of water content. Soil Sci. Soc. Am. J. 70, 2008-2016.

Rokitko, P.V., Romanovskaya, V.A., Malashenko, Y.R., Chernaya, N.A., Gushcha, N.I., Mikheev, A.N., 2003. Soil drying as a model for the action of stress factors on natural bacterial populations. Microbiology 72, 756-761.

Romanovskaya, V.A., Sokolov, I.G., Rokitko, P.V., Chernaya, N.A., 1998. Effect of radioactive contamination on soil bacteria in the 10-km zone around the Chernobyl Nuclear Power Plant. Microbiology 67, 226-231.

Rosén, K., Oborn, I., Lonsjo, H., 1999. Migration of radiocaesium in Swedish soil profiles after the Chernobyl accident, 1987-1995. J. Environ. Radioact. 46, 45-66.

Rosén, K., Zhong, W., Martensson, A., 2005. Arbuscular mycorrhizal fungi mediated uptake of 137Cs in leek and ryegrass. Sci. Total Environ. 338, 283-290.

Sanchez, A.L., Parekh, N.R., Dodd, B.A., Ineson, P., 2000. Microbial component of radiocaesium retention in highly organic soils. soil Biol. Biochem. 32, 2091-2094.

Santrucková, H., Bird, M.I., Elhottová, D., Novák, J., Picek, T., Simek, M., Tykva, R., 2005. Heterotrophic fixation of CO_2 in soil. Microb. Ecol. 49, 218-225.

Scheffer, F., Schachtschabel, P., Blume, H.-P., 2002. Lehrbuch der Bodenkunde. Spektrum Akademischer Verlag, Berlin.

Schimmack, W., Schultz, W., 2006. Migration of fallout radiocaesium in a grassland soil from 1986 to 2001: Part I: Activity-depth profiles of ^{134}Cs and ^{137}Cs. Sci. Total Environ. 368, 853-862.

Schlegel, H.-G., Fuchs, G., Eitinger, T., 2007. Allgemeine Mikrobiologie. Thieme, Stuttgart.

Shaw, G., 2007. Radionuclides in Forest Ecosystems. Elsevier, Amsterdam.

Shaw, L.J., Burns, R.G., 1998. Biodegradation of 2,4-D in a noncontaminated grassland soil profile. J. Environ. Qual. 27, 1464-1471.

Simonoff, M., Sergeant, C., Poulain, S., Pravikoff, M.S., 2007. Microorganisms and migration of radionuclides in environment. C. R. Chim. 10, 1092-1107.

Smith, A.E., Aubin, A.J., 1991. Metabolites of ^{14}C-2,4-dichlorophenoxyacetic acid in Saskatchewan soils. J. Agric. Food Chem. 39, 2019–2021.

Smith, A.E., Aubin, A.J., 1994. Loss of enhanced biodegradation of 2,4-D and MCPA in a field soil following cessation of repeated herbicide applications. Bull. Environ. Contam. Toxicol. 53, 7-11.

Smith, S.E., Read, D.J., 2008. Mycorrhizal symbiosis (third edition), 3 ed. Academic Press, Amsterdam ; Boston.

Smolders, E., Van den Brande, K., Merckx, R., 1997. Concentrations of ^{137}Cs and K in Soil Solution Predict the Plant Availability of ^{137}Cs in Soils. Environ. Sci. Technol. 31, 3432–3438.

Soulas, G., 1993. Evidence for the existence of different physiological groups in the microbial community responsible for 2,4-D mineralization in soil. Soil Biol. Biochem. 25, 443 - 449.

Stahl, P.D., Parkin, T.B., 1996. Relationship of soil ergosterol concentration and fungal biomass. Soil Biology and Biochemistry 28, 847-855.

Steiner, M., Linkov, I., Yoshida, S., 2002. The role of fungi in the transfer and cycling of radionuclides in forest ecosystems. J. Environ. Radioact. 58, 217-241.

Stott, D.E., Martin, J.P., Focht, D.D., Haider, K., 1983. Biodegradation, stabilization in humus, and incorboration into soil biomass of 2,4-D and chlorocatechol carbons. Soil Sci. Soc. Am. J. 47, 66-70.

Strandberg, M., Johansson, M., 1998. ^{134}Cs in heather seed plants grown with and without mycorrhiza. J. Environ. Radioact. 40, 175-184.

Stroh, K., 2006. Pflanzenschutzmittel in der Umwelt. UmweltWissen. Bayerisches Landesamt für Umwelt, http://www.lfu.bayern.de/umweltwissen/doc/uw_52_pflanzenschutzmittel_umwelt.pdf.

Tester, C.F., 1988. Role of soil and residue microorganisms in determining the extent of residue decomposition in soil. Soil Biol. Biochem. 20, 915-919.

Thiry, Y., Myttenaere, C., 1993. Behaviour of radiocaesium in forest multilayered soils. J. Environ. Radioact. 18, 247-257.

Thompson, J.P., 1990. Soil sterilization methods to show VA-mycorrhizae aid P and Zn nutrition of wheat in vertisols. Soil Biol. Biochem. 22, 229-240.

Tiedje, J.M., Alexander, M., 1969. Enzymatic cleavage of ether bond 2,4-dichlorophenoxyacetate. J. Agric. Food. Chem. 17, 1080 - 1084.

Torsvik, V., Goksoyr, J., Daae, F.L., 1990. High diversity in DNA of soil bacteria. Appl. Environ. Microbiol. 56, 782-787.

Tugay, T., Zhdanova, N.N., Zheltonozhsky, V., Sadovnikov, L., Dighton, J., 2006. The influence of ionizing radiation on spore germination and emergent hyphal growth response reactions of microfungi. Mycologia 98, 521-527.

UNSCEAR, 1988. Sources and Effects of Ionizing Radiation. United Nations Scientific Committee on the Effects of Atomic Radiation Vienna.

UNSCEAR, 1996. Sources and Effects of Ionizing Radiation. United Nations Scientific Committee on the Effects of Atomic Radiation Vienna.

UNSCEAR, 2000. Sources and Effects of Ionizing Radiation. United Nations Scientific Committee on the Effects of Atomic Radiation Vienna.

Unterweger, M.P., 2002. Half-life measurements at the National Institute of Standards and Technology. Appl. Radiat. Isot. 56, 125-130.

van Bergeijk, K.E., Noordijk, H., Lembrechts, J., Frissel, M.J., 1992. Influence of pH, soil type and soil organic matter content on soil-to-plant transfer of radiocesium and -strontium as analyzed by a nonparametric method. J. Environ. Radioact. 15, 265-276.

Vinichuk, M.M., Johanson, K.J., 2003. Accumulation of Cs-137 by fungal mycelium in forest ecosystems of Ukraine. J. Environ. Radioact. 64, 27-43.

Volkmer, M., 2007. Basiswissen Kernenergie. Informationskreis Kernenergie, Berlin.

Wang, W.J., Baldocka, J.A., Dalala, R.C., Moody, P.W., 2004. Decomposition dynamics of plant materials in relation to nitrogen availability and biochemistry determined by NMR and wet-chemical analysis. Soil Biol. Biochem. 36, 2045-2058.

Ward, T.E., 1985. Characterizing the aerobic and anaerobic microbial activities in surface and subsurface soils. Environ. Toxicol. Chem. 4, 727-737.

Wauters, J., Elsen, A., Cremers, A., Konoplev, A.V., Bulgakov, A.A., Comans, R.N.J., 1996. Prediction of solid/liquid distribution coefficients of radiocaesium in soils and sediments. Part one: A simplified procedure for the solid phase characterisation. Appl. Geochem. 11, 589-594.

West, A.W., Grant, W.D., Sparling, G.P., 1987. Use of ergosterol, diaminopimelic acid and glucosamine contents of soils to monitor changes in microbial populations. Soil Biology and Biochemistry 19, 607-612.

Wiklander, L., 1964 Uptake, adsorption and leaching of radiostrontiumnext term in a lysimeter experiment. Soil Science 97, 168–172.

Wilson, M.A., 1987. NMR Techniques and Applications in Geochemistry and Soil Chemistry, 1 ed. Pergamon Press, Oxford.

Yablokov, A.V., Nesterenko, V.B., Nesterenko, A.V., 2009. Chernobyl consequences of the catastrophe for people and the environment. New York Acad. of Sciences, Boston, Mass.

Yoshida, S., Muramatsu, Y., 1998. Concentrations of alkali and alkaline earth elements in mushrooms and plants collected in a Japanese pine forest, and their relationship with ^{137}Cs. J. Environ. Radioact. 41, 183-205.

Young, E., Oh, D.J., 2006. 2,4-Dichlorophenoxyacetic Acid Pathway Map. University of Minnesota, http://umbbd.msi.umn.edu/2,4-d/2,4-d_map.html.

Zhdanova, N.N., Tugay, T., Dighton, J., Zheltonozhsky, V., Mcdermott, P., 2004. Ionizing radiation attracts soil fungi. Mycological Research 108, 1089-1096.

Zhu, Y.G., Shaw, G., 2000. Soil contamination with radionuclides and potential remediation. Chemosphere 41, 121-128.

Zhu, Y.G., Smolders, E., 2000. Plant uptake of radiocaesium: a review of mechanisms, regulation and application. J. Exp. Bot. 51, 1635-1645.

8 Appendix

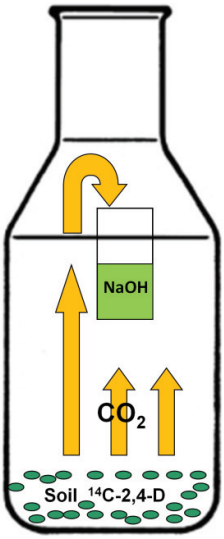


Figure 8.1: Schematic design of a soil microcosm. The microorganisms in the soil degrade the ^{14}C-labeled substrate and release $^{14}CO_2$. This will be absorbed in the NaOH and measured in the liquid scintillation counter (LSC). The measured $^{14}CO_2$ divided by the totally applied ^{14}C radioactivity of the wheat straw results in the mineralization rate.

Figure 8.2: Soil microcosm array. a) Soil microcosms in the incubator. b) Soil microcosms tempered in a heated water bath. During incubation the microcosms were shaded.

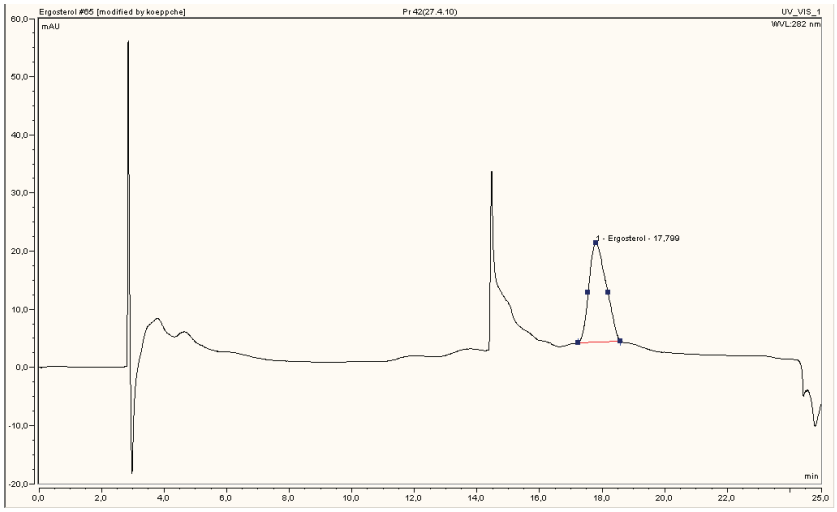


Figure 8.3: Example chromatogram of Ergosterol measured with HPLC-UV/VIS.

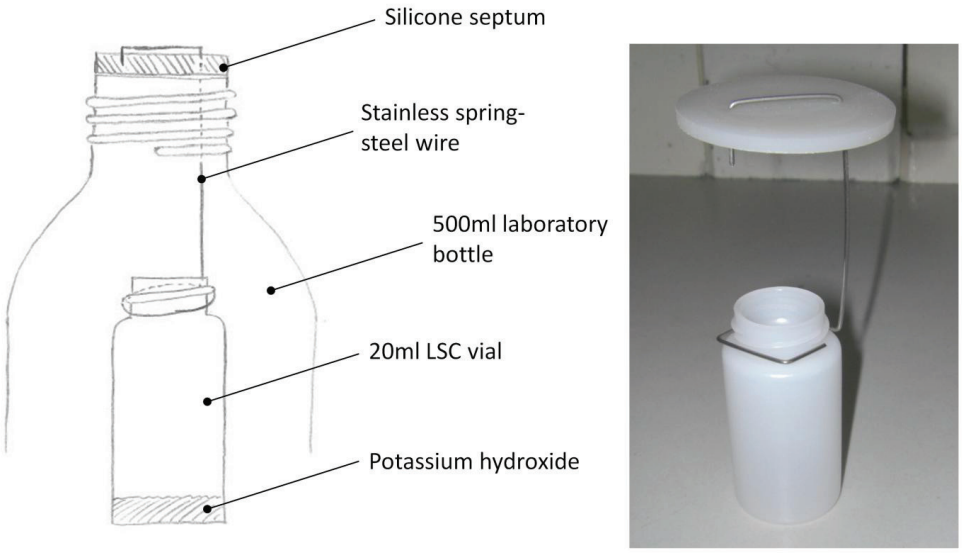


Figure 8.4: Improved construction of the NaOH traps used in the experiments. The silicone septum seals the microcosms and fixes the stainless steel spring wire. CO_2 traps: LSC vial filled with NaOH for absorbing the $^{14}CO_2$ evolving from the degradation of ^{14}C-labeled substrate in the soil microcosms. The traps were replaced each second day by new ones, filled with scintillation cocktail and measured for absorbed radioactivity in the LSC.

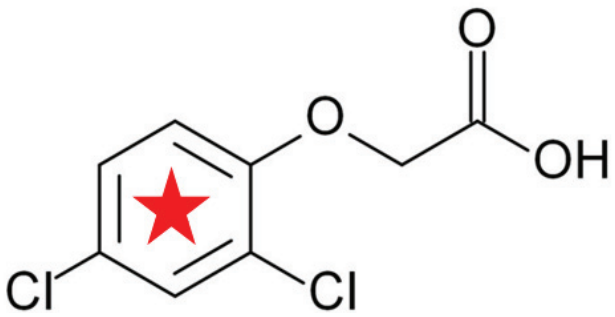


Figure 8.5: ^{14}C-U-ring-labeled 2,4-Dichlorophenoxyacetic acid (2,4-D). Red star: ^{14}C labeling position in the phenyl ring

Danksagung

Zunächst möchte ich ganz herzlich Peter Burauel danken, dass er meine Arbeit betreut hat und mir bei den diversen Problemen bei der Einstellung geholfen hat. Harry Vereecken möchte ich für die Unterstützung und die Halbjahresgespräche danken. Besonders die “half-year reports” waren sehr nützlich.

Zu Beginn der Promotion bereitete mir der Umgang mit radioaktiven Substanzen besondere Kopfzerbrechen, hierbei wurde ich sehr von Werner Mittelstaedt, Martina Krause und Ulrike Langen unterstützt. Hierfür und die vielen anderen Hilfestellungen im Labor ein herzliches Dankeschön!

Für meine Fahrt in die Ukraine möchte ich mich bei Jürgen Höbig von S und ebenfalls bei Werner bedanken. Die drei Tage vor Weihnachten haben viel Spaß gemacht und mich doch irgendwie weiter gebracht.

Für die Unterstützung bei den gefühlt 1000 gegossenen DGGE-Gelen möchte ich mich ganz besonders bei Sirgit Kummer, Sascha Sokolowsky und Wolfgang Tappe bedanken.

Bei Wolfgang möchte ich mich auch für die vielen Gespräche (auch jenseits der Arbeit) bedanken. Ich denke du konntest mir bei vielen Problemen helfen, oder zumindest die Augen öffnen! Nicht zuletzt einen großen Dank für die vielen Urlaubstips!

Dann möchte ich mich noch bei der Mittagsrunde bedanken, dadurch war die Mittagspause nie langweilig und ging meistens viel zu schnell rum. Und gelernt hat man auch meistens was. Danke dafür an Thomas Muckenheim, Daniela Kasel, Christian Lichters, Jürgen Höltkemeier, Fabian Syberberg, Sascha, Wolfgang und Sirgit.

Für die viele Ablenkung im Büro ☺ möchte ich mich ganz besonders bei meinen Bürokollegen Chengliang Li, Fabian, David Vonberg und Khalid Mehmood bedanken.

Stephan Köppchen und Anne Berns möchte ich für die Analysen mit der HPLC und dem NMR danken, Stephan ausserdem für die tolle Zusammenarbeit als Strahlenschutzbeauftragter.

Many thanks for the great time at the Sveriges lantbruksuniversitet in Uppsala to Stefan Bengtsson, Mykhailo Vinichuk and Klas Rosén.

Für die Hilfe, insbesondere bei den englischen Texten und für manchen freundschaftlichen Rat möchte ich mich bei Nicolai Jablonowski und Niklas Rademacher bedanken.

Herrn Prof. F. Führ danke ich für die hilfreichen Gespräche und die Korrektur der ersten Publikationen.

Ein ganz großer Dank gilt meiner Familie, die mich immer unterstützt hat und meiner lieben Freundin Cristina, die so manch aufmunterndes Wort aussprechen musste!

Band / Volume 148
IEK-Report 2011. Klimarelevante Energieforschung
(2012), ca. 250 pp.
ISBN: 978-3-89336-808-2

Band / Volume 149
IEK-Report 2011. Climate-Relevant Energy Research
(2012), ca. 250 pp.
ISBN: 978-3-89336-809-9

Band / Volume 150
Netzintegration von Fahrzeugen mit elektrifizierten Antriebssystemen in bestehende und zukünftige Energieversorgungsstrukturen (2012)
ISBN: 978-3-89336-811-2

Band / Volume 151
Stratospheric ClOOCl chemistry at high solar zenith angles
O. Suminska-Ebersoldt (2012), VI, 126 pp
ISBN: 978-3-89336-817-4

Band / Volume 152
Keramiken und Keramikkombinationen zur Feinstpartikelabscheidung mit Hilfe thermisch induzierter Potentialfelder und Elektronenemissionen
D. Wenzel (2012), XXV, 155 pp
ISBN: 978-3-89336-820-4

Band / Volume 153
Bildung von sekundären Phasen bei tiefengeologischer Endlagerung von Forschungsreaktor-Brennelementen – Struktur- und Phasenanalyse
A. Neumann (2012), 329 pp
ISBN: 978-3-89336-822-8

Band / Volume 154
Coupled hydrogeophysical inversion for soil hydraulic property estimation from time-lapse geophysical data
M. Cho Miltin (2012), xi, 79 pp
ISBN: 978-3-89336-823-5

Band / Volume 155
Tiefentschwefelung von Flugturbinenkraftstoffen für die Anwendung in mobilen Brennstoffzellensystemen
Y. Wang (2012), 205 pp.
ISBN: 978-3-89336-827-3

Band / Volume 156
Self-consistent modeling of plasma response to impurity spreading from intense localized source
M. Koltunov (2012), V, 113 pp.
ISBN: 978-3-89336-828-0

Band / Volume 157
Phosphorsäureverteilung in Membran-Elektroden-Einheiten dynamisch betriebener Hochtemperatur-Polymerelektrolyt-Brennstoffzellen
W. Maier (2012), VI, 105 pp.
ISBN: 978-3-89336-830-3

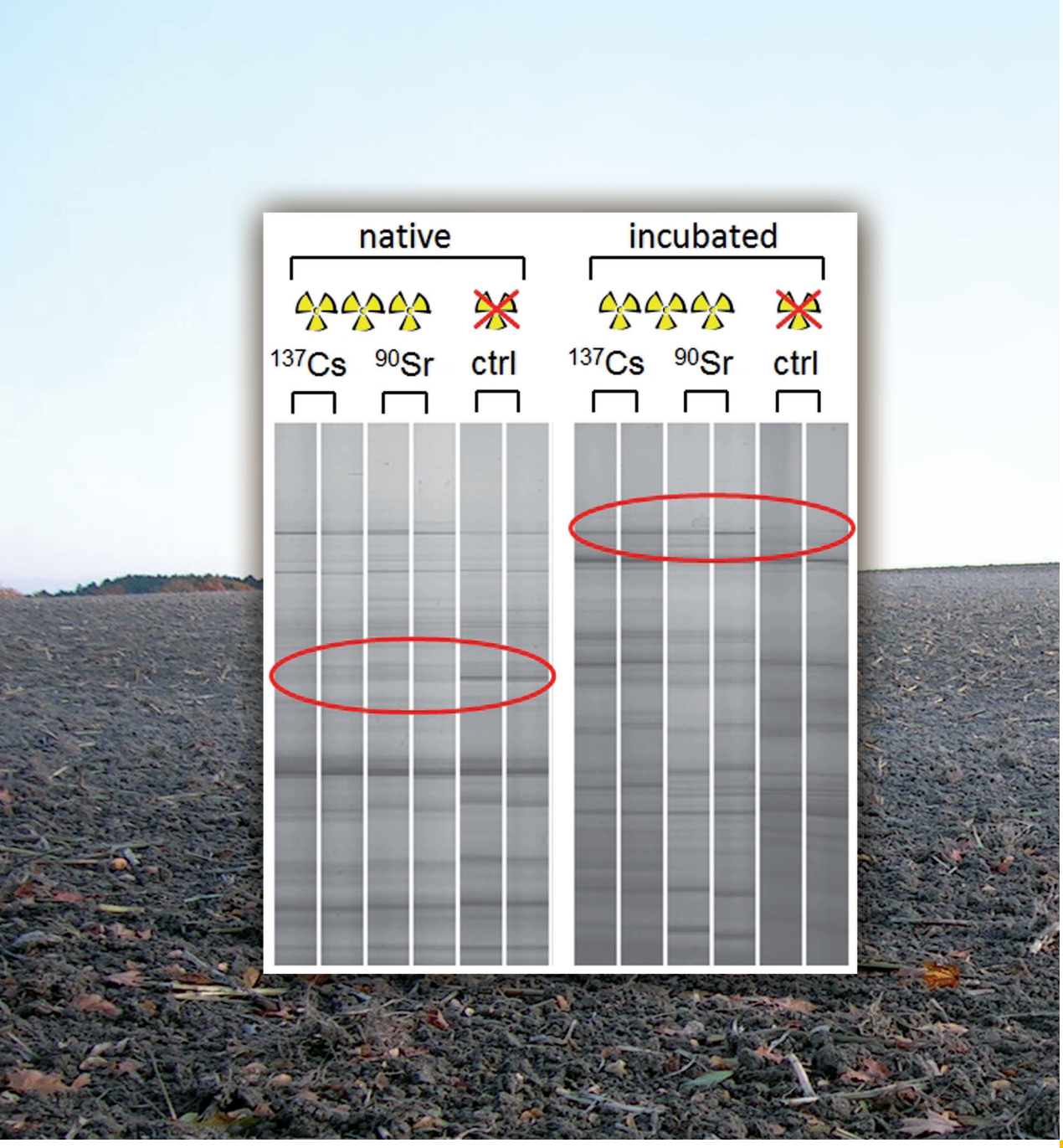
Band / Volume 158
Modellierung und Simulation von Hochtemperatur-Polymerelektrolyt-Brennstoffzellen
M. Kvesic (2012), ix, 156 pp.
ISBN: 978-3-89336-835-8

Band / Volume 159
Oxidation Mechanisms of Materials for Heat Exchanging Components in CO_2/H_2O-containing Gases Relevant to Oxy-fuel Environments
T. Olszewski (2012), 200 pp.
ISBN: 978-3-89336-837-2

Band / Volume 160
Ice Crystal Measurements with the New Particle Spectrometer NIXE-CAPS
J. Meyer (2013), ii, 132 pp.
ISBN: 978-3-89336-840-2

Band / Volume 161
Thermal Shock Behaviour of Different Tungsten Grades under Varying Conditions
O. M. Wirtz (2013), XIV, 130 pp.
ISBN: 978-3-89336-842-6

Band / Volume 162
Effects of ^{137}Cs and ^{90}Sr on structure and functional aspects of the microflora in agricultural used soils
B. Niedrée (2013), XII, 92 pp.
ISBN: 978-3-89336-843-3



Energie & Umwelt / Energy & Environment
Band / Volume 162
ISBN 978-3-89336-843-3

JÜLICH
FORSCHUNGSZENTRUM