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Diffusion processes of soluble organic substances in soil and their effect on ecological processes

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"... Measurements of Diffusion are a Holy Grail Requiring Noble Knights Who Dedicate Their Lives to the Quest."

E. L. Cussler

Abstract

Diffusion of dissolved organic matter (DOM) is one of the key processes in the soil ecosystem. At small scales diffusion is often the predominant transport process. As a result it influences organic matter turnover and soil building processes. Although the importance of diffusion processes in soil is known since a long time there are only very few studies which focus on diffusion of DOM in soil as a research subject. Investigation of DOM diffusion in soil is challenging due to soil DOM being a complex mixture of mostly unidentified substances, due to its high turnover rates, and due to its role in several processes.

There is a number of open questions regarding diffusion of soil DOM which need to be answered. Not much is known about the intrinsic diffusion characteristics of natural DOM. Of particular interest is whether the very diverse molecules which are part of the DOM lead to a comparable diversity of diffusion coefficients and consequently to fractionation of DOM during diffusion. Another important question is the extent of diffusion processes following the introduction of heterogeneities such as organic matter sources or microbial sinks into soil. This question needs to be answered to explain the small scale distribution of organic matter and microorganisms in soil.

Three model experiments were conducted to gain specific knowledge regarding these questions. The first experiment addressed intrinsic diffusion properties of DOM. A device consisting of two compartments divided by a glass frit was used to study diffusion of DOM from soil solutions and extracts of barley plants in free aqueous solution. Fractions of DOM were distinguished by their fluorescence properties. Interestingly diffusion coefficients of DOM were found to be relatively high, i. e. they were similar to the tabulated diffusion coefficient of the sucrose molecule. It follows that soil DOM can at the most contain minor amounts of humic macromolecules. Diffusion coefficients of different DOM fractions were found to be similar. Only tryptophan-like fluorophores in plant extract diffused significantly faster with diffusion coefficients identical to the theoretical value of the amino acid tryptophan. In soil solutions these fluorophores did not diffuse faster than other DOM fractions. It is concluded that diffusion alone does not lead to strong fractionation of soil DOM.

In two other model experiments source induced and sink induced diffusion of DOM in soil were investigated together with microbial degradation of organic matter. ^{14}C-labeling was employed to distinguish carbon fluxes in soil with high resolution. Source induced diffusion was investigated following introduction of ^{14}C-labeled plant litter and ^{14}C-labeled microbial biomass respectively into soil. Diffusion of a ^{14}C-labeled pesticide in soil towards microbial degrader hot-spots was investigated as an example of sink induced diffusion. In both experiments diffusion lead to ^{14}C profiles in the millimeter to centimeter scale. Diffusion out of litter in soil lead to a ^{14}C-labeling of living microbes which was constant independent of distance one month after start of the experiment. Diffusion of the pesticide towards degraders competed against formation of bound residues. Results indicate that pesticide derived carbon was transported out of the degrading hot-spots. Both experiments showed that although diffusion fluxes are small they can influence organic matter and microbial populations around heterogeneities in distances up to the centimeter scale.

Zusammenfassung

Die Diffusion gelöster organischer Substanz (DOM) ist einer der Schlüsselprozesse im Bodenökosystem. Auf kleinen Skalen ist Diffusion oft der dominierende Transportprozess. Daher beeinflusst sie den Umsatz organischer Substanz und Bodenbildungsprozesse. Obwohl die Wichtigkeit von Diffusionsprozessen in Böden seit Langem bekannt ist, gibt es kaum Studien, die sich auf Diffusion von DOM als Forschungsgegenstand konzentrieren. Die Untersuchung der Diffusion von DOM im Boden ist eine Herausforderung, da DOM im Boden eine komplexe Mischung größtenteils nicht identifizierter Substanzen ist, seine Umsatzraten sehr hoch sind und es an vielen Prozessen im Boden beteiligt ist.

Es bestehen eine Reihe offener Fragen bezüglich DOM in Böden. Über die immanenten Diffusionseigenschaften von natürlicher DOM ist nur wenig bekannt. Von besonderem Interesse ist, ob die hohe molekulare Diversität von DOM zu einer vergleichbaren Diversität der Diffusionskoeffizienten und daher zur Fraktionierung während der Diffusion führt. Eine weitere wichtige Frage ist das Ausmaß der Diffusionsprozesse infolge der Einbringung von Heterogenitäten in Böden, beispielsweise Quellen organischer Substanz oder mikrobielle Senken, in den Boden. Diese Frage muss beantwortet werden, um die kleinräumige Verteilung organischer Substanzen und von Mikroorganismen im Boden zu verstehen.

Im Rahmen dieser Arbeit wurden drei Modelexperimente durchgeführt. Das erste Experiment befasste sich mit den immanenten Diffusionseigenschaften von DOM. Ein Versuchsaufbau bestehend aus zwei durch eine Glasfritte getrennten Behältern wurde verwendet, um Diffusion von DOM aus Bodenlösungen und Gerstenpflanzenextrakten in freier wässriger Lösung zu untersuchen. Die DOM-Fraktionen wurden anhand ihrer Fluoreszenzeigenschaften unterschieden. Interessanterweise waren die Diffusionskoeffizienten der DOM relativ hoch, d. h. ähnlich wie der tabellierte Diffusionskoeffizient des Sucrosemoleküls. Daraus folgt, dass die DOM höchstens geringe Mengen an Huminmakromolekülen enthalten kann. Diffusionskoeffizienten der unterschiedlichen DOM-Fraktionen unterschieden sich wenig. Nur Tryptophanähnliche Fluorophoren im Pflanzenextrakt diffundierten signifikant schneller, wobei die Diffusionskoeffizienten mit dem theoretischen Wert für die Aminosäure Tryptophan identisch waren. In Bodenlösungen diffundierten diese Fluorophoren nicht schneller als andere DOM-

Fraktionen. Daraus wird geschlussfolgert, dass Diffusion alleine nicht zu einer starken Fraktionierung von DOM im Boden führt.

In zwei weiteren Modellexperimenten wurden quellenverursachte und senkenverursachte Diffusion zusammen mit mikrobiellem Abbau von organischen Substanzen untersucht. ^{14}C-Markierung wurde eingesetzt, um Kohlenstoffflüsse im Boden hochauflösend verfolgen zu können. Quellenverursachte Diffusion wurde nach Zugabe von ^{14}C-markierter Pflanzenstreu beziehungsweise ^{14}C-markierter mikrobieller Biomasse zu Boden untersucht. Die Diffusion eines Pestizids im Boden in Richtung von Hotspots mikrobieller Abbauer wurde als Beispiel von senkenverursachter Diffusion untersucht. In beiden Experimenten führte Diffusion zu ^{14}C-Profilen auf Millimeter- bis Zentimeterskalen. Die Diffusion im Boden führte von der Streu ausgehend zu einer ^{14}C-Markierung der lebenden Mikroorganismen, die einem Monat nach der Streuzugabe unabhängig von der Distanz konstant war. Die Pestiziddiffusion in Richtung der zugefügten Abbauer konkurrierte mit der Entstehung gebundener Rückstände. Die Ergebnisse weisen darauf hin, dass aus dem Pestizid stammender Kohlenstoff aus den abbauenden mikrobiellen Hotspots heraus transportiert wurde. Beide Experimente zeigten, dass, obwohl Diffusionsflüsse nur klein sind, diese dennoch die organische Substanz und mikrobielle Populationen in Entfernungen bis zur Zentimeterskala um Heterogenitäten beeinflussen können.

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List of symbols

A	absorbance
C	concentration
C_{mic}	concentration of microbial carbon in soil
CM	cumulative mineralization
D	diffusion coefficient
D_a	apparent diffusion coefficient
D_{aq}	diffusion coefficient in free aqueous solution
D_e	effective diffusion coefficient
I	fluorescence intensity
J	mass flux
K_d	linear sorption coefficient
K_f	Freundlich coefficient
R	retardation factor
R_0	solute radius
S_L	(liquid) solubility
T	temperature
V	volume

a	chemical activity
a	fluorophore concentration loading in PARAFAC
b	fluorophore excitation loading in PARAFAC
c	fluorophore emission loading in PARAFAC
d_s	solid density
k_b	Boltzmann's constant
k_{EC}	ectraction factor for chloroform fumigation extraction
m	empirical parameter in Archie's law
n	form exponent in van Genuchten model
n_f	Freundlich exponent
q	concentration of adsorbed substance in soil
t	time
x	position

α	probability of false positive
α	form factor in van Genuchten model
β	calibration factor of diffusion cells

ε	porosity
ε_t	transport-through porosity
θ	volumetric water content
θ_{sat}	volumetric water content at saturation
μ	viscosity
π	circle constant
ρ	bulk density
τ	constrictivity
Ψ	matrix potential

List of acronyms

2-OH-Mono	1-[4-(2-hydroxypropan-2-yl)phenyl]-3-methylurea (2-hydroxy-monodesmethyl-isoproturon)
ASE	accelerated solvent extraction
Dides-IPU	1-[4-(propan-2-yl)phenyl]urea (didesmethyl-isoproturon)
DOC	dissolved organic carbon
DOM	dissolved organic matter
DON	dissolved organic nitrogen
DOP	dissolved organic phosphorous
DOS	dissolved organic sulfur
EDB	ethanolamine and diethylenglycolmonobuthylether (V : V = 1 : 1)
EEM	excitation emission matrix
EEMS	excitation emission matrix fluorescence spectroscopy
HPLC	high performance liquid chromatography
IC	inorganic carbon
IPU	1,1-dimethyl-3-[4-(propan-2-yl)phenyl]urea (isoproturon)
IUPAC	International Union of Pure and Applied Chemistry
MATLAB	a numerical computing software suite
Monodes-IPU	1-methyl-3-[4-(propan-2-yl)phenyl]urea (monodesmethyl-isoproturon)
NDIR	nondispersive infrared
NMR	nuclear magnetic resonance
PARAFAC	parallel factor analysis
PMT	photomultiplier tube
PTFE	polytetrafluorethylen
SE	standard error

SOM	soil organic matter
SPE	solid phase extraction
TC	total carbon
TNb	total nitrogen bound
TOC	total organic carbon
UD-SOM	undissolved soil organic matter
WHC	water holding capacity

Chapter 1

Introduction and background

Soil organic matter (SOM) is one of the most important parts of the soil ecosystem. It is involved in soil building processes, facilitates sorption processes, and serves as microbial substrate. SOM heavily influences crop yield from agricultural soils. Furthermore storage of carbon in the SOM pool is considered a key mechanism of carbon sequestration on a global scale.

The most active and consequently most labile form of SOM is dissolved organic matter (DOM). SOM has to be transformed into DOM in order to serve as a food source of microorganisms. DOM also plays an important role in transport of organic matter in soil, particularly on small scales.

Although transport processes of organic matter in soil have been investigated intensively, in the past most studies focused exclusively on convective transport. Diffusion of DOM in soil received far less attention. Convective transport is a key component of soil-hydrosphere interactions. However, most in-situ processes take place in microsites and thus diffusion is the predominant transport mechanism which controls source-sink systems. Important examples of such systems are degradation of SOM and sorption processes. In order to better understand the soil ecosystem more knowledge about diffusion of DOM in soil and particularly its interaction and interdependencies with other processes in soil is needed.

1.1 Dissolved organic matter in the soil ecosystem

Dissolved organic matter (DOM) is the most active and labile form of SOM (McGill et al., 1986; Corvasce et al., 2006). Although it accounts only for a small fraction of SOM DOM plays an essential role in the soil ecosystem (Zsolnay, 1996). DOM is a crucial part of carbon and nitrogen cycles by providing soil microbes with substrate (Kalbitz, Schmerwitz, Schwesig

and Matzner, 2003; Marschner and Kalbitz, 2003; Cookson et al., 2005). Furthermore it is considered a major controlling factor in mineral weathering (Raulund-Rasmussen et al., 1998; Egli et al., 2001), soil formation (Lundström et al., 1995; Jansen et al., 2005), and pollutant transport (Madhun et al., 1986; Magee et al., 1991; Boesten, 1993; Li et al., 2005).

By convention DOM is operational defined as the organic matter of a solution that can pass through a filter membrane with a pore size of around 0.45 µm (Sholkovitz, 1976; Zsolnay, 2003; Corvasce et al., 2006). Therefore DOM includes most of the colloidal organic matter. The composition of DOM is governed by its sources and subsequent modification by microbial activity and (bio)chemical reactions. The main sources of DOM are plant litter, root exudates, soil fauna and microorganisms, and the undissolved soil organic matter (UD-SOM) pool which exceeds the DOM pool by two orders of magnitude and more (Zsolnay, 1996). An additional source in agriculturally used soils are organic fertilizers. Agricultural practice heavily influences SOM and consequently DOM, e. g. by crop rotation, tillage, and fertilization (Zsolnay and Görlitz, 1994; Embacher et al., 2007).

Although a number of powerful analytical techniques, e. g. nuclear magnetic resonance (NMR) spectroscopic techniques, is available (Kögel-Knabner, 2000), fully analyzing DOM compounds is a tedious task and a complete chemical characterization is impossible (Kalbitz, Solinger, Park, Michalzik and Matzner, 2000). The main reason for this is that DOM contains poorly defined macromolecules, particularly humic substances. To characterize these substances multiple techniques have to be combined. Since most of these techniques require specific sample preparation, this is work and time consuming. Hence, quality of DOM is generally analyzed only to the extent necessary for a specific research question. Nearly always applied are elemental analyzes, especially measurement of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON), and more seldom dissolved organic phosphorous (DOP) and dissolved organic sulfur (DOS). As they are fast and inexpensive spectroscopic methods such as UV/VIS spectroscopy and fluorescence spectroscopy are frequently used to assess DOM quality (Senesi, 1990; Zsolnay et al., 1999; Kalbitz, Geyer and Geyer, 2000).

Processes involved in transformation of soil organic matter (SOM) between pools (figure 1.1) are transport (convective transport, diffusion), physicochemical (sorption, precipitation, dissolution), chemical (protonation, complexation), or biological (decomposition, mineralization) processes. Fresh organic material is ultimately decomposed by the edaphon and undergoes humification processes. Furthermore its soluble constituents are leached or diffuse out

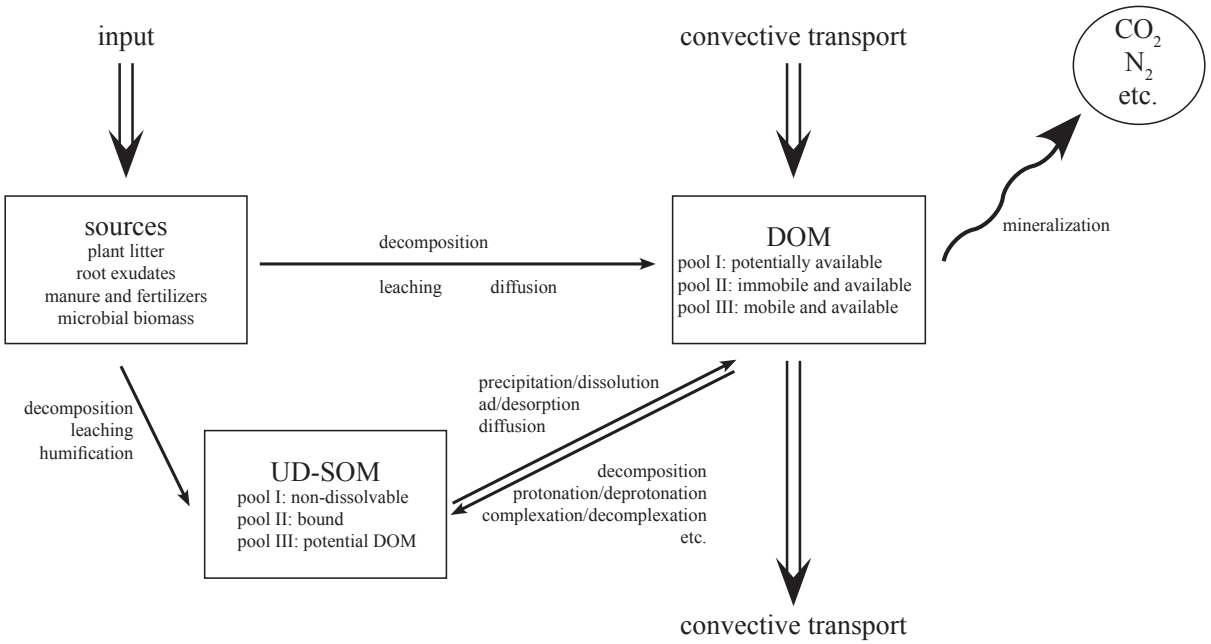


Figure 1.1: Conceptual model of the pools and processes involved in the formation of DOM (adapted from Kalbitz, Solinger, Park, Michalzik and Matzner, 2000; Zsolnay, 2003). The model takes into account three main pools: a source pool of fresh organic matter, a DOM pool and an undissolved soil organic matter (UD-SOM) pool. Processes leading to transfer between pools are given.

of the source material, consequently becoming part of the DOM pool. The remaining, generally more persistent compounds such as cellulose, lignin, and humic substances build the undissolved soil organic matter (UD-SOM) pool together with sorbed and precipitated soluble substances and remains of microbial biomass. There is a wide range of interactions between the DOM and the UD-SOM pool. Some of these transformation processes take place nearly continuously to a certain extent, e. g. decomposition, while others are initiated by changes in soil condition, e. g. precipitation/dissolution by changes in pH or water content.

The DOM pool (figure 1.1) may be divided into three subpools (Zsolnay, 2003), which are defined by the pore space. DOM I is located in pores of a size below 0.2 µm. This subpool is not subjected to convective transport and it can only be transported by diffusion. It is believed to be not accessible to microbes (von Lützow et al., 2006) and therefore might only be degraded by abiotic processes or possibly exoenzymes. As a result it is the most stable part of the DOM pool and only potentially available to microorganisms. DOM II, which is DOM in pores of size 0.2 µm to 6 µm, can be transported by convection although diffusion is still mostly the dominant transport mechanism (Zsolnay, 1996), which results in relatively low mobility. DOM II is accessible to microorganisms as is DOM III, which is the part of the DOM pool in pores of a size above 6 µm. Transport of DOM III is dominated by convection (Zsolnay, 1996), so it is the

most mobile DOM. Transfer between the DOM subpools takes place by diffusion, for instance strong microbial activity might deplete DOM II and III, which could then lead to diffusion of DOM out of the fine pores and restoration of the subpools.

It is feasible to divide the UD-SOM pool (figure 1.1) into subpools with regard to how easy it can be transformed into DOM. Substances that can be considered to be insoluble in soil solution, e. g. cellulose and lignin, build subpool UD-SOM I. This organic material has to undergo (bio)chemical changes such as decomposition or protonation to become soluble and thereby part of the potential DOM (UD-SOM III). Substances of subpool UD-SOM II are bound to or encased by insoluble organic or other soil material, e. g. clay minerals. Aggregates or covalent bindings have to be broken for these substances to become (potentially) dissolved. UD-SOM III comprises all organic substances which are adsorbed or precipitated and can dissolve easily and fast if the DOM concentration decreases.

1.2 Bioavailability of DOM

Although bioavailability is a fundamental parameter of all degradation processes in soil, there is no widely accepted clear definition of bioavailability and many varying definitions are used (Semple et al., 2004, 2007). In this work the definition of Marschner and Kalbitz (2003) is adopted: "Bioavailability [in soil] describes the potential of microorganisms to interact with DOM." Higher organisms are not considered here. Bioavailability depends on several factors. Obviously microorganisms able to degrade (or at least susceptible to) a substance have to be present for interactions between microorganisms and the substance to be possible (Bosma et al., 1997). If contact between microbes and substance is not restricted bioavailability is determined by microbial species and chemical properties, e. g. hydrophobicity, of the substance (ISO 17402, 2008-06).

DOM may be divided into at least three groups with regard to intrinsic bioavailability (Marschner and Kalbitz, 2003). Compounds, which can be degraded via common metabolic pathways and therefore are preferentially utilized by a large number of microbial species (Volk et al., 1997; Amon et al., 2001), are part of the labile DOM (Lynch, 1982; van Hees et al., 2005). It is assumed that simple carbohydrate monomers (i. e., glucose, fructose), low molecular organic acids, amino acids, amino sugars and low-molecular-weight proteins belong in this group (Lynch, 1982; Qualls and Haines, 1992; Koivula and Hänninen, 2001; van Hees et al., 2005). A more stable fraction is believed to consist of polysaccharides and other plant or microbially

derived compounds or degradation products that require special microbial tools for degradation. These substances are most likely only degraded if the labile pool is exhausted or mostly by K-strategists (Marschner and Kalbitz, 2003). Lignin degradation products and compounds strongly altered by preceding degradation steps, commonly called humic substances (Saiz-Jimenez, 1996), are believed to be degraded extremely slowly and thus build a recalcitrant DOM fraction (Marschner and Kalbitz, 2003).

A further important parameter of bioavailability is the chemical activity, or more precisely chemical potential, of a substance in vicinity of the microorganisms (Reichenberg and Mayer, 2006). Chemical activity influences the rates of physicochemical processes such as diffusion and (bio)chemical reactions. It is a function of concentration, substance and solvent. For instance, to achieve the same activity of a hydrophobic compound in a hydrophilic solvent, e. g. a soil solution, as in a more hydrophobic solvent, e. g. microbial biomass, a lower concentration would be needed in the former than in the latter. Chemical activity can be approximately expressed as (Ferguson, 1939):

$$a \approx \frac{C_{\text{free}}}{S_L} \tag{1.1}$$

where a is chemical activity, C_{free} is freely dissolved concentration, and S_L is (liquid) solubility. A system is in equilibrium if activity is identical in all parts of the system. This can lead to very differing concentrations at equilibrium if different solvents, such as soil solution and microbial biomass, are involved.

A fundamental aspect of bioavailability in soil is accessibility which accounts for the effect of unsaturated conditions and the soil matrix on bioavailability. It is believed that microorganisms might use nearly exclusively dissolved substances as substrate (Marschner and Kalbitz, 2003; Kalbitz, Schwesig, Schmerwitz, Kaiser, Haumaier, Glaser, Ellerbrock and Leinweber, 2003). The amount of dissolved substances is reduced by precipitation, sorption processes and binding to UD-SOM or soil minerals (Guggenberger et al., 1994; Jandl and Sollins, 1997). The access of microbes to DOM in soil can be further hampered by occlusion of DOM in small pores (see section 1.1) or UD-SOM (McCarthy et al., 2008). Such occluded DOM is generally only accessible by diffusion out of the micropores. Therefore diffusion characteristics of DOM influence its accessibility (Chung et al., 1993).

There are mainly two possibilities to estimate bioavailability. (i) The more accurate is measurement of degradation or mineralization, i. e. soil respiration (Birch, 1958; Kalbitz, Schmerwitz, Schwesig and Matzner, 2003). If experimental conditions are adjusted to optimum for

microbial activity, bioavailability is limiting and can therefore be determined by measuring degradation or mineralization. (ii) The other approach correlates extractability with bioavailability (Reichenberg and Mayer, 2006). This is possible because accessibility, more precisely mass transfer, and not the microorganisms are in most cases the critical factor in bioavailability (Bosma et al., 1997). Extractions with aqueous solvents are used to determine the amount of substance accessible in the short-term. This fraction of SOM is referred to as water extractable organic matter (Zsolnay, 2003). Harsher extraction procedures, e. g. using high temperature or compound specific solvents, can be applied to measure long-term accessibility, but are not widely used in studies of SOM. Pools with different accessibility and availability can be determined by sequential extractions (Spaccini et al., 2000).

1.3 Heterogeneities in soil

Living conditions of microorganisms in soil habitats are characterized by a water-unsaturated pore space with strongly varying water potentials. Such an environment does not favor microbial strategies which lead to strong motility. The vast majority of soil microorganisms does not swim free in soil solution, but is attached to pore walls as biofilms and microcolonies (Or et al., 2007). Although one gram of soil contains up to one billion microorganisms (Rosello-Mora and Amann, 2001) less than 1 % of the inner soil surface is colonized (Young and Crawford, 2004). Spatial divers environments together with low mobility and low population densities cause a high biodiversity (Reichenbach et al., 2007) and a very heterogeneous distribution of soil microorganisms (Young and Crawford, 2004) and functions (Gonod et al., 2003) on all scales, particularly the microscale (Ettema and Wardle, 2002). Although soil microbes are mostly found close to their substrates the spatial patterns of the distributions of microorganisms and SOM do not match exactly. Other factores such as competition, predators, or oxygen supply might cause conditions which lead to spatial seperation of microorganisms from their food source. In such a case diffusion of DOM sustains microbial activity. Furthermore microbial activity produces degradation products, which are transported out of microbial hot-spots and may be used as substrate by other microorganisms.

1.4 Diffusion in soil

Diffusion is the transport of a substance from high chemical potential to low chemical potential due to the thermal motion of particles. In many cases, particularly if diffusion takes place in an ideal solution at constant temperature, the gradient of chemical potential can be approximated by the concentration gradient (Atkins, 1998).

Diffusion processes in soil are mostly assumed to follow Fick's second law of diffusion (Grathwohl, 1998) which in the one dimensional case is the partial differential equation (Crank, 1970)

$$\frac{\partial C}{\partial t} = \frac{\partial C}{\partial x}\left(D\frac{\partial C}{\partial x}\right) \tag{1.2}$$

where C is concentration, t is time, x is position, and D is diffusion coefficient. Under steady-state conditions this equation simplifies to Fick's first law of diffusion (Fick, 1855):

$$J = -D\frac{\partial C}{\partial x} \tag{1.3}$$

where J is mass flux.

To solve equation 1.2 for concentration numerous numerical and analytical procedures (e. g., Crank, 1970; Carslaw and Jaeger, 1959) depending on initial and boundary conditions can be applied. In any case the value of the diffusion coefficient is needed. This value depends on the substance in question and the matrix where diffusion takes place.

Diffusion coefficients of a number of common substances in aqueous solution can be found in the literature (e. g., Cussler, 1997) or calculated from other tabulated quantities such as molar conductivity (Lide, 1999-2000). It is also possible to use approximations like the Stokes-Einstein equation (Einstein, 1905) and its adaptations (Cussler, 1997) or the Wilke-Chang correlation (Wilke and Chang, 1955) to calculate diffusion coefficients from known parameters of substance and solvent.

There are two key methods for the **measurement of diffusion coefficients in liquids** (Cussler, 1997). An inexpensive, very accurate method using a robust experimental system is the diaphragm cell method (described in section 2.6.1). This method allows to study diffusion of mixtures if appropriate analyses are applied. The second method is the Taylor dispersion method. Here a solvent is pumped with laminar flow through a long tube, a sharp pulse of the solute is injected at one end, and the dispersion of the pulse measured on the other end of

the tube. This system is easy to build and easy to handle. However, it is usually only practical for the study of binary mixtures. Other methods for the measurement of diffusion coefficients are, e. g., dynamic light scattering and voltammetry (Pinheiro et al., 1996). These methods pose strict requirements on sample characteristics and are not suitable for complex sample matrices.

Diffusion in a porous medium is affected by the pore system of the medium. On the macroscopic scale this is taken into account by defining an effective diffusion coefficient (Grathwohl, 1998):

$$D_e = \frac{D_{aq}\varepsilon_t\delta}{\tau} \tag{1.4}$$

The transport-through porosity ε_t is the porosity which accounts for pores available for transport. It can be smaller than the overall porosity because of too small, dead end or blind pores. Constrictivity τ accounts for drag resulting from higher viscosity of pore solution which is caused by proximity to pore walls (Cussler, 1997). Small pores, especially with pore sizes of the same order of magnitude as the size of the diffusing particles, and narrow pore throats result in a smaller constrictivity value. The constrictivity factor is significantly different from 1 if pore diameters are below 10 nm (Hu and Wang, 2003). Tortuosity τ describes pore geometry and accounts for meandering pores.

Both constrictivity and tortuosity may be calculated from theoretical considerations (van Brakel and Heertjes, 1974; Epstein, 1989) or empirical equations (Grathwohl, 1998). They can not be measured directly, but conservative tracer experiments can be used to determine these parameters (Boving and Grathwohl, 2001). Often they are simply treated as modeling parameters.

In unsaturated materials the pore space which is available for diffusion of a nonvolatile substance is approximately expressed as the volumetric water content θ and the effective diffusion coefficient becomes (Schaefer et al., 1995)

$$D_e = \frac{D_{aq}\theta\delta}{\tau} \tag{1.5}$$

It should be noted that tortuosity and constrictivity of a porous medium change with the water content as a function of water tension (Hu and Wang, 2003).

Inter-particle diffusion which is diffusion in inter-particle pores, and intra-particle diffusion which is diffusion through the particles of a porous medium can be considered separately

(Millington and Shearer, 1971). However, in most cases it is feasible to use an overall effective diffusion coefficient (Cioranescu and Donato, 1999).

Sorption processes slow down diffusional transport. If sorption takes place this may be taken into account by defining an apparent diffusion coefficient

$$D_a = \frac{D_e}{R} \tag{1.6}$$

where R is a retardation factor. In case of linear sorption ($q = K_d C$) the apparent diffusion coefficient can be calculated (Hu and Wang, 2003) as

$$R = 1 + \frac{K_d \rho}{\theta} \tag{1.7}$$

where K_d is the linear sorption coefficient and ρ is bulk density. If sorption is nonlinear the retardation factor and therefore the apparent diffusion coefficient becomes concentration dependent. As a consequence solving equation 1.2 becomes a very complicated task if sorption of the solute follows a nonlinear law.

There are various experimental setups for the **determination of diffusion coefficients in saturated porous media** (for details see Shackelford, 1991). For unsaturated conditions two alternatives exist. The **half-cell method** (Schaefer et al., 1995) brings two compartments of the medium, one with the substance and one without the substance (or with a lower concentration), into contact. After an appropriate time-span the medium is sampled along the concentration gradient and the diffusion coefficient calculated from the concentration profile (see section 2.6.3). The method is time consuming and a high number of samples has to be analyzed. As a result the obtained diffusion coefficient is usually statistically firm. The **infinite-sink method** (e.g., Warncke and Barber, 1972a) brings a strong solid phase adsorbent into contact with the medium containing the substance that is studied. After sufficient time the adsorbent is sampled and analyzed for the substance. The adsorbed mass allows calculation of the diffusion coefficient. For statistically firm results numerous experimental replicates are needed. The challenge of this method is to find a suitable adsorbent which works in contact with the porous medium. This can be difficult, particularly in experiments with soil.

In the last century **diffusion of pollutants in soil** materials has been studied extensively. Many studies focused on diffusion through fine grained barrier materials (Gillham et al., 1984; Shackelford, 1991; Johnson et al., 1989; Rowe et al., 1988, and others) which are used in envi-

ronmental engineering, e. g. as enclosures of landfills. Other studies investigated diffusion of inorganic ions (Boving and Grathwohl, 2001; Warncke and Barber, 1972a,b; Barraclough and Tinker, 1981, 1982; Porter et al., 1960; Brown et al., 1964, and others) in soil. Inorganic ions were often chosen as a research subject in diffusion studies because analytical procedures have been established for a long time. Furthermore most inorganic ions are not subjected to degradation processes. Plant nutrients were also a frequent subject of diffusion studies. Romkens and Bruce (1964) studied diffusion of nitrate and Olsen et al. (1965) studied diffusion of phosphorous in soil. Olsen and Kemper (1968) wrote a very comprehensive paper on movement of nutrients to plant roots which focuses mainly on diffusion.

Studies about **diffusion of natural DOM in soil** are rare and in these studies diffusion is always investigated together with other processes, especially with degradation by microbes. The main difficulty in studying transport of natural organic matter is that DOM is a mostly unknown mixture of substances (see section 1.1). A common solution to this problem that allows to follow fluxes in soil is the use of labeling techniques. ^{14}C labeling of plants (Sauer et al., 2006) as well as naturally stable isotope labeled plant litter (Poll et al., 2006) have been employed in experiments. ^{14}C labeling enables detection of very low concentrations and allows to observe concentration profiles far from source. However, intensive safety procedures have to be applied. Furthermore only fresh organic matter can be ^{14}C labeled at reasonable expense. To produce aged ^{14}C labeled soil organic matter a long time labeling experiment over several years would be needed. Stable isotope techniques allow the use of naturally different ^{13}C to ^{12}C ratios of DOC originating from C3- and C4-plants. Theoretically they can be used to study diffusion of aged organic matter. However, detection limits of stable isotope measurement are much higher in comparison to radioactive isotopes which makes quantitative evaluation of diffusion experiments difficult. So far all of the very few studies on diffusion of natural organic matter in soil have been carried out with fresh organic material.

1.5 Degradation of litter in soil

Crop residues and other plant litter are not only the primary source of SOM (Thurman, 1984; Chantigny, 2003), but they also heavily influence SOM quality. Furthermore, litter input can influence soil properties such as pH (Marschner and Noble, 2000; Xu et al., 2006) and soil aggregation (Helfrich et al., 2008; Denef and Six, 2006).

Basically plant litter consists of a lignin fraction, a hemicellulose and cellulose fraction, and a soluble fraction (Trinsoutrot et al., 2000; Adair et al., 2008). The initial phase of litter degradation is strongly related to the soluble fraction of litter (Williams and Gray, 1974; Stevenson, 1986) which contains easily degradable compounds like carbohydrates (e. g., free sugars) and organic acids (e. g., amino acids) (Stevenson, 1982, 1986; Thurman, 1984). For instance, Marschner and Noble (2000) observed a loss of DOC in soil amended with plant litter from several plant species during incubation. This decline was in very good correlation with cumulative CO_2-evolution which shows that nearly exclusively DOM was used as microbial substrate during the initial phase of litter degradation. The soluble compounds of plant litter appreciably differ in quantity and quality according to plant species (Thurman, 1984; Zsolnay, 1996; Marschner and Noble, 2000). In later stages of degradation recalcitrant macromolecules like cellulose and finally even lignin are decomposed and their breakdown products (e. g., sugars, amino acids, phenolic compounds), which are mostly soluble, are mineralized (Stevenson, 1986).

The process of litter degradation leads to production of microbial cells, which after death are further degraded (Grandy and Neff, 2008). Proteins and simple biochemical compounds from these cells (e.g., sugars and amino acids) decompose quickly, but cell walls and some melanins are more recalcitrant (Stevenson, 1986). Because all stages of litter degradation involve dissolved substances, they are accompanied by diffusion of DOM out of the litter. This results in a volume of soil where microbial activity and SOM are directly influenced by the plant litter. This volume is called the detritusphere (Poll et al., 2008). Diffusion and degradation of the substances have opposing effects on the size of the detritusphere. Gaillard et al. (2003) found that in incubation experiments 23 % to 33 % of the mineralization of cereal straw took place in soil outside the zone containing particulate litter.

Generally substances with high diffusion coefficients are also very labile in soil. Therefore the size of the detritusphere depends largely on the interaction of microorganisms and diffusion (Poll et al., 2006).

1.6 Pesticides as a model of SOM

Pesticides are needed in agricultural practice to ensure crop yields and food security. Due to the fact that they pose a risk to public health (e. g., Charlier et al., 2003) and the environment (e. g., Relyea, 2003) these organic chemicals are of scientific interest since a long time. As a

result there exists much data and knowledge about the fate of pesticides in soil (Arias-Estévez et al., 2008), although some crucial questions are still not completely answered.

Pesticides and other organic chemicals in soil are part of the SOM pool, though they account for only up to a small percentage (Thurman, 1984). In principle they are subjected to the same processes as natural SOM (Stevenson, 1986; Bollag et al., 1992). They are adsorbed, bound to SOM or clay minerals, precipitate, dissolve, are leached, transported, and degraded (Stevenson, 1986; Kozak, 1996). Many pesticides or their degradation products share characteristics with natural molecules of SOM, particularly with more recalcitrant natural substances. Partial degraded pesticides can even be precursor molecules for the synthesis of humic substances (Stevenson, 1982). Therefore principles found in studies of pesticides in soil often also apply to natural organic matter in soil (e. g., Xing and Pignatello, 1998).

Pesticides have some advantages that make them easier to study than natural SOM. They (and their early degradation products) are distinct substances with known chemical structure. Effective analytical methods are generally available (Andreu and Pico, 2004). Furthermore it is relatively easy to apply radioactive or stable isotope labeling to these substances, which makes it possible to follow the fate of pesticide derived carbon in soil (e. g., Wanner et al., 2005).

Another advantage of pesticides in experimental studies is that it is often possible to isolate microbial communities or even single strains that specifically degrade a pesticide (e. g., Grundmann et al., 2007; Holtze et al., 2008). This allows experimental setups that are generally not possible with natural organic matter.

Because of the aforementioned advantages it is feasible to use pesticides as a model of SOM substances. Results of pesticide studies in soil can illuminate general principles of SOM transformations and interactions with soil and soil microorganisms.

1.7 Objectives of this thesis

A basic parameter of diffusion in soil is the diffusion characteristics of the substances that are transported. This thesis addresses diffusion characteristics of natural DOM in free aqueous solution as there is still a profound gap of knowledge in this research area. The following hypothesis was tested:

H_0: Fractions of DOM which show different stability against degradation in soil have different diffusion coefficients in free aqueous solution.

From the hypothesis it would follow that soil DOM is in its nature very prone to fractionation according to its stability. If the hypothesis could be disproved such a fractionation would not follow directly from diffusion but could only be caused by diverse sorption properties of the soil pore surfaces.

The presented thesis specifically aims to improve the knowledge about the role of diffusion of DOM in the context of heterogeneities in the soil ecosystem. The study focused specifically on the interdependencies between diffusion processes, microbial activity and sorption processes in soil. Two basic systems were studied:

Source induced diffusion: A strong source of DOM creates concentration gradients which lead to diffusion. The most important example of this kind of system is litter in soil. Soluble litter compounds are transported out of the litter into the adjacent soil where they are degraded. Thus, degradation of litter, which had been added to soil as a spatially confined source of organic matter, was studied for this thesis.

Sink induced diffusion: Degradation of a homogeneously distributed organic substance in microbial hot-spots creates a diffusion flux towards the microorganisms. This kind of system evolves after addition of mineral fertilizer or pesticide to a soil. In the scope of this thesis an experiment was conducted on degradation of a herbicide in soil.

A special focus has been put on the microbial biomass in vicinity of sinks and sources, in particular on how much carbon which is accumulated in the biomass originates from the diffusing substance.

Chapter 2

Materials and methods

Three model experiments were conducted in the course of this thesis. In the first experiment diffusion characteristics of natural DOM in aqueous diffusion was studied. In the other two experiments source induced diffusion and sink induced diffusion, respectively, were investigated to study the diffusion mediated effect of heterogeneities introduced to soil on soil organic matter and microbial biomass.

2.1 Characteristics of soil materials

The Ap horizons of four agricultural sites were sampled for model experiments. In the "free diffusion" experiment (see section 2.6 for a description of the experiments) two Cambisols from the research farm of the Helmholtz Zentrum München in Scheyern (Bavaria) were used. One was sampled shortly after harvest and one shortly after organic fertilizer addition and plowing. The "source diffusion" experiment was conducted with the former of these Cambisols and an Haplic Arenosol which was sampled in Neumarkt (Bavaria). In the "sink diffusion" experiment an Humic Cambisol from Kelheim (Bavaria) was used. Important soil properties are given in table 2.1.

For the "free diffusion" experiment (see section 2.6) undisturbed soil cores were sampled and pore water extracts were obtained by centrifugation (see section 2.9.2). For the other experiments disturbed samples were sieved to 2 mm and homogenized. The Haplic Arenosol was stored at -20 °C, the other soils at 4 °C. All soils were preincubated one week at ambient temperature in the laboratory before the start of an experiment, the Haplic Arenosol after 3 days thawing at 4 °C.

Table 2.1: Properties of soil materials from Ap horizons used in the experiments.

identifier	Cambisol 1	Cambisol 2	Cambisol 3	Arenosol
location	Scheyern 1	Scheyern 2	Kelheim	Neumarkt
soil type	Cambisol	Cambisol	Humic Cambisol	Haplic Arenosol
soil texture[1]				
clay (< 2 µm) %	19.3 (19.0)	(27.9)	11	4
silt (2 - 63 µm) %	40.4 (39.7)	(57.1)	19	8
sand (63 - 2000 µm) %	40.3 (39.6)	(16.2)	70	88
solid density g cm^3	2.68	2.75	n.d.	n.d.
bulk density g cm^3	1.16	1.27	n.d.	n.d.
pH ($CaCl_2$)	7.1	5.7	6.4	5.2
carbonate-C %	0.1	n.d.	0.1	< 0.05
org C %	1.4	1.4	1.1	0.9
total N %	0.17	0.15	0.12	0.08
used in experiment[2]	"free diffusion", "source diffusion"	"free diffusion"	"sink diffusion"	"source diffusion"

[1] numbers in parentheses: soil prior to sieving, else 2 mm sieved soil
[2] see section 2.6 for a description of the experiments
n.d.: not determined

Figure 2.1 shows the water retention curves of the four soils used in the experiments. The values for soils "Cambisol 3" and "Arenosol" were measured at the Institute for Soil Science of Technical University Munich (Schroll et al., 2006). Data for soil "Cambisol 2" was kindly provided by Claudia Zimmermann (Chair of Soil Protection and Recultivation, Brandenburg University of Technology). The following procedure (DIN ISO 11274, 2001-01) was used:

The soil was air-dried and sieved to 2 mm. Soil samples were pressed into small (10 cm^3) metal rings to achieve a soil density of 1.3 g cm^{-3} and then rewetted to yield water holding capacity (WHC). The samples were transfered to a sand/kaolin box (08.02 Eijkelkamp, Netherlands) where water potentials from -10 kPa to -50 kPa were stepwise applied until equilibrium which was checked gravimetrically. Subsequently higher water potentials up to -1500 kPa were applied in a pressure extractor (1500 F1, Eijkelkamp, Netherlands) with a ceramic plate. At each step the mass of the samples was determined and the water content calculated. For soil "Cambisol 2" undisturbed samples (volume: 80 cm^3) were used and only treated in a pressure extractor.

A water retention model (simplified from van Genuchten, 1980) was fitted to the data:

$$\theta = \theta_{sat}\left[1 + (\alpha|\Psi|)^n\right]^{\frac{n-1}{n}} \tag{2.1}$$

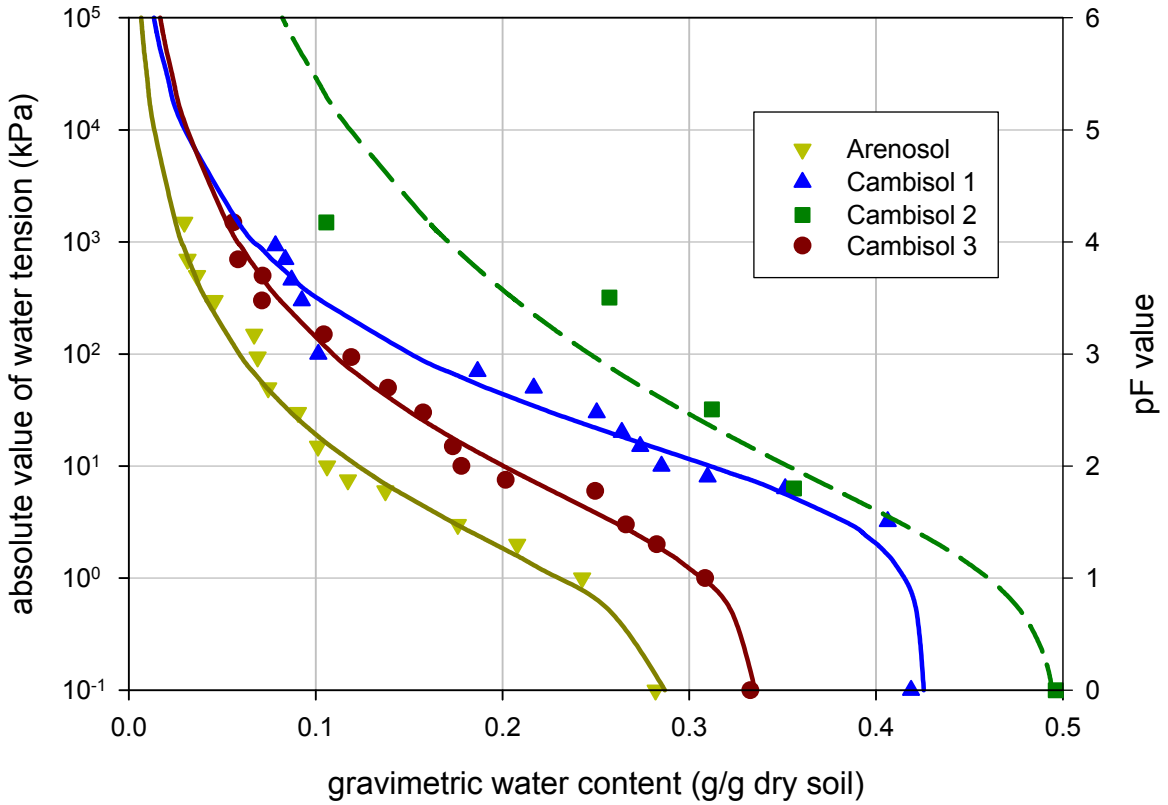


Figure 2.1: Relationship between water tension and water content for the four soils used in the experiments. Dots show measured values; lines represent corresponding fitted soil - water retention curves. The dashed line is given as a rough estimation for soil "Cambisol 2" as the number of measured values is insufficient for a good regression.

where θ is volumetric water content, θ_{sat} is volumetric water content at saturation, Ψ is matrix potential, and α and n are shape parameters. Because of the low number of data points the fit to soil "Cambisol 2" data gives only a rough estimate of the water retention curve.

2.2 ^{14}C-labeling of barley plants

Barley (*Hordeum vulgare* L.) was grown in a desiccator with an atmosphere containing ^{14}C-labeled CO_2 (figure 2.2) to produce ^{14}C-labeled plant biomass to be used in the "source diffusion" experiment (see section 2.6).

Barley seeds were sterilized prior to germination with the following treatment (von Rad, 2006):

1. 2 min immersion in 80 % ethanol followed by flushing with sterile water
2. 20 min immersion in aqueous solution of 5 % sodium hypochlorite and 0.2 % Tween 20 (Merck Bioscience, UK) followed by flushing with sterile water
3. 3 d storage in antibiotic-antimycotic solution (streptomycin sulphate, penicillin G sodium) at 4 °C
4. flushing twice with sterile water

For germination the seeds were then put on plates (2.2 g L^{-1} Murashige&Skoog medium [Duchefa, The Netherlands], 10.0 g L^{-1} saccharose, 3.5 g L^{-1} Gerlrite [Duchefa, The Netherlands]) under sterile conditions and kept in the dark at 20 °C for four days. Afterwards the most vital seedlings were selected for ^{14}C-labeling.

Washed sand (Sakret, Germany) was heated to 550 °C for 12 hours and stored at 250 °C. The cooled down sand was put into a desiccator, 2.2 g L^{-1} Murashige&Skoog medium was added, and the barley seedlings were put in. The closed desiccator was placed under a light source (Osram L 18W/25, Osram, Germany) in a hood and connected to the head space of an Erlenmeyer flask which contained 160 °C hot phosphoric acid (figure 2.2). Using a peristaltic pump ^{14}C-labeled sodium carbonate (51.6 g L^{-1}, 74 MBq) in 0.1 M NaOH solution was added to the flask with a flow rate of 1.7 mL h^{-1} to produce ^{14}C-labeled CO_2 for a continuous ^{14}C-labeling of the barley plants. Overpressure was avoided with an expandable bag connected to the system. For a 5 hour night period the peristaltic pump was stopped and the light switched off by a timer. Every morning the system was aerated with ambient air over absorption tubes with ethanolamine and diethylenglycolmonobuthylether (V : V = 1 : 1) (EDB) to collect the ^{14}C-labeled CO_2 which had not been assimilated by the plants.

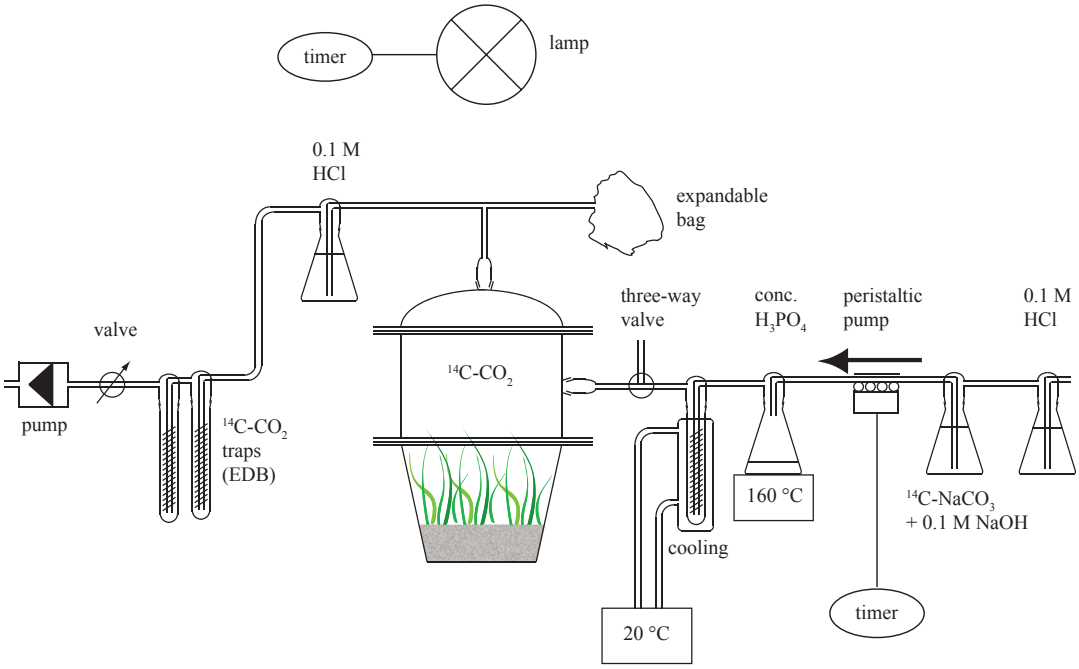


Figure 2.2: Setup for ^{14}C-labeling of barley plants. The plants grow in an atmosphere with ^{14}C-CO_2 which is produced continuously by dripping ^{14}C-carbonate solution into hot phosphoric acid. An expandable bag avoids overpressure. Both ^{14}C-CO_2 production and illumination are controlled by timers. Once a day the system is aerated with ambient air.

After four weeks the barley plants (including roots) were harvested, air dried, and ground with a ball mill (MM 2, Retsch, Germany). The resulting powder had a radioactive label of 2000 Bq mg^{-1}. It was stored at -20 °C.

2.3 ^{14}C-labeling of microbial biomass

A soil microbial community was grown with ^{14}C-labeled glucose as a substrate to produce ^{14}C-labeled microbial biomass to be used in the "source diffusion" experiment.

A soil extract was produced by extracting soil "Cambisol 1" with tap water at a soil : extractant ratio of 1 : 1 (m: m) by shaking for 30 min. The suspension was then filtered (prewashed 595 1/2 filter papers, Whatman, Schleicher&Schuell) and autoclaved (20 minutes at 121°C). A soil extract nutrient medium was prepared with a composition as shown in table 2.2 and adjusted to pH 7.1 by addition of HCl and NaOH. ^{14}C-labeled glucose was added to achieve a radioactive label of 1.85 MBq in 1000 mL medium. 3 g soil "Cambisol 1" were extracted with 30 mL autoclaved water by shaking for 30 min. After a sedimentation time of 15 min 375 µL of the supernatant were added as inoculum to 250 mL medium. The culture was prepared in quadruplicate. The cultures were aerated continuously on a horizontal shaker at lab temperature and CO_2 trapped in absorption tubes with EDB (see appendix A, figure A.2 for mineralization results).

Table 2.2: Composition of soil extract nutrient medium

compound	**concentration**
soil extract	50 % (volume)
glucose	3.0 g L^{-1}
Bacto pepton	0.3 g L^{-1}
$Na_2HPO_4 \cdot 12H_2O$	1.5 g L^{-1}

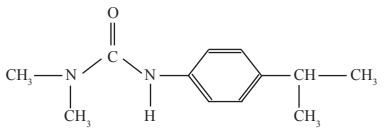
After 9 days 59.3 % of the applied ^{14}C-glucose had been mineralized. The cultures were then centrifuged with 7000×g. Subsequently the resulting biomass pellet was washed twice with a 0.9 % NaCl solution, autoclaved, and dried at 50 °C. Because of the low amount of substance it was not possible to grind it in a ball mill and mortar and pestle had to be used instead. This resulted in a biomass material which was not as fine-grained as the barley biomass (section 2.2). Furthermore there was not enough biomass to determine its specific radioactivity. It was tried to determine the radioactive label of the material mixed with soil (as there was a small remain after starting the "source diffusion" experiment) but results showed that the value was not representative.

2.4 Isoproturon

Isoproturon {1,1-dimethyl-3-[4-(propan-2-yl)phenyl]urea} was used as a model substance in the "sink diffusion" experiment. It is a widely used herbicide in cereal production across Europe. As a result it is frequently detected in surface waters and groundwaters (e.g., Spliid and Køppen, 1998; European Commision, 2002; Ormad et al., 2008). The main properties of isoproturon are shown in table 2.3. In this study ^{14}C uniformly ring labeled isoproturon was used. It was mixed with the formulation components of the commercial available product Arelon according to specification of the pesticide producer Agrevo (Hoechst, Germany).

Table 2.3: Chemical-physical properties of the ^{14}C labeled isoproturon (Schroll et al., 2006).

vapor pressure at 20 °C	2.8 - 8.1	Pa
water solubility at 20 °C	70.2	$mg\,L^{-1}$
Henry's law constant at 22 °C	$1.46 \cdot 10^{-5}$	$Pa\,m^3\,mol^{-1}$
molecular mass	206.3	$g\,mol^{-1}$
partition coefficient (log K_{OW})	2.5	
half-life under field conditions	approx. 60 - 300	d
concentration in Arelon	5.07	$mg\,mL^{-1}$
final specific radioactivity	0.69	$MBq\,mg^{-1}$
radioactive purity	95	%



2.5 Standard experimental conditions

All soil experiments were conducted at a water tension of -15 kPa. This water potential is expected to be optimal for aerobic microbial activity in soils (Miller and Johnson, 1964; Ilstedt et al., 2000; Schroll et al., 2006) and therefore was chosen as standard condition. Soil experiments were carried out at lab temperature (22 °C). Experiments on DOM of soil solutions were carried out at 4 °C in order to inhibit decomposition by microorganisms.

2.6 Experimental designs

Three model experiments were conducted. A short overview of the experiments is given in table 2.4.

Table 2.4: Aim and principle of the three diffusion experiments.

	aim	principle
"free diffusion" experiment	investigate diffusion velocities of DOM fractions	donor and acceptor solutions are divided by a glass frit and concentration changes measured
"source diffusion" experiment	investigate source induced diffusion of DOM caused by degradation of heterogeneously distributed substrate	^{14}C-labeled plant or microbial biomass at one end of a soil tube results in spatial concentration gradients
"sink diffusion" experiment	investigate sink induced diffusion of DOM caused by degradation of homogeneously distributed substrate in microbial hot spots	a layer of microbial hot spots in the middle of a soil tube supplemented with a ^{14}C-labeled pesticide results in spatial concentration gradients

In the "free diffusion" experiment diffusion of soil dissolved organic matter (DOM) and fresh DOM from barley plant biomass in free aqueous solutions was investigated. The aim of this experiment was to determine diffusion coefficients of chemically different fractions of DOM and, particularly, the resulting change in composition of the DOM mixture as a function of distance from source. This leads to knowledge on the fundamental diffusional properties of those fractions and the interactions between them independent of soil properties.

In the "source diffusion" experiment diffusion of DOM in soil was investigated in conjunction with degradation of fresh organic matter (plant litter and microbial biomass). The experimental results provide insight as to how a discrete source of organic matter induces diffusion of well metabolizable substrates in soil. Source induced diffusion, its interaction with degradation, and the effect of the source on organic matter and microbial biomass in the source's vicinity were studied with this experiment.

In the "source diffusion" experiment the diffusion processes resulting from the degradation of a pesticide by microbial hot spots in soil were studied. Here the pesticide as a microbial substrate was initially evenly distributed in soil and the activity of discrete microbial hot spots created concentration gradients which then induced diffusion of the substrate to the microbes. Sink induced diffusion, its interaction with degradation, and the effect of the sink on organic matter and microbial biomass in the sink's vicinity were studied with this experiment.

2.6.1 Diffusion of DOM and inorganic nitrogen in free solution ("free diffusion" experiment)

Diffusion cells (figure 2.3, after Cussler, 1997) were constructed to study diffusion in free aqueous solution. The devices consist of two reservoirs divided by a glass frit (pore size 10 - 16 µm, item no. 15404, ROBU, Germany). In the lower reservoir a receptor solution is filled in. A donor solution which contains the substance or mixture to be studied is then filled in the upper reservoir. After an appropriate time span (usually several days) both halves of the cell are sampled and concentrations measured. The diffusion coefficient D_{aq} may then be calculated (Cussler, 1997):

$$D_{aq} = \frac{1}{\beta t} \cdot \ln \frac{C_{1,0}}{C_{1,t} - C_{2,t}} \tag{2.2}$$

where β is a calibration factor describing the physical dimensions of the device, t is the time span of the experiment, $C_{1,0}$ is initial concentration in the donor reservoir, and $C_{1,t}$ and $C_{2,t}$ are the final concentrations in donor and receptor reservoir, respectively. The calibration factor was determined by conducting the experiment repeatedly with urea and then calculating the calibration factor from the tabulated diffusion coefficient at 25 °C of $1.38 \cdot 10^{-5}$ cm^2 s^{-1} (Cussler, 1997). To check the calibration the diffusion coefficient of benzoic acid was determined. The measured value of $1.01 \cdot 10^{-5}$ cm^2 s^{-1} $\pm$ $0.02 \cdot 10^{-5}$ cm^2 s^{-1} standard error (SE) was in excellent agreement with the tabulated value at 25 °C of $1.00 \cdot 10^{-5}$ cm^2 s^{-1} (Cussler, 1997). Both urea and benzoic acid were quantified with a TOC analyzer (see section 2.10.1).

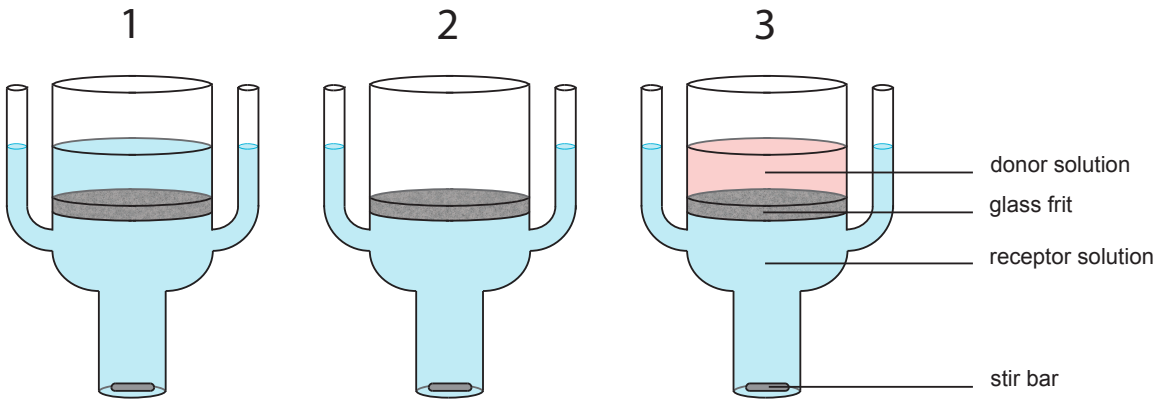


Figure 2.3: Diffusion cells consisting of a donor reservoir and a receptor reservoir separated by a glass frit. In the first step both reservoirs are filled with receptor solution to saturate the frit. In the second step the donor reservoir is emptied and in the third step filled with donor solution. After an appropriate time span both reservoir are sampled and diffusion coefficients can be calculated.

The experiments were conducted at 4 °C to inhibit microbial degradation processes. To transform the results to other temperatures validity of the Stokes-Einstein equation is assumed:

$$D_{aq} = \frac{k_B T}{6\pi\mu R_0} \tag{2.3}$$

where k_b is Boltzmann's constant, T is absolute temperature, π is the circle constant, μ is viscosity of the solvent, and R_0 is solute radius. Since viscosity of water is the only factor that depends on temperature it follows that:

$$D_{T_1} \cdot \frac{\mu_1}{T_1} = D_{T_2} \cdot \frac{\mu_2}{T_2} \tag{2.4}$$

The viscosity of water at 25 °C and 4 °C is 0.8901 mPa s and 1.5667 mPa s, respectively (Lemmon et al., 2005).

Soil solutions which were extracted by centrifugation (see section 2.9.2) from two different soils ("Cambisol 1" and "Cambisol 2", see subsection 2.1) and an extract (see section 2.9.1) from barley plants were investigated in this experiment. The experiments were conducted in 5 replicates. Mass balances were calculated. If a mass balance was outside the range [85 %, 115 %] the corresponding diffusion coefficient was discarded for evaluation.

Cleaning procedure for diffusion cells

Because of the small pore size of the diffusion cell glass frits an extensive cleaning procedure had to be applied. The following steps were carried out after each use of a diffusion cell:

1. flushing twice with double deionized water (Milli-Q Plus, Millipore)
2. filling with 2 M HCl for 12 h
3. flushing again with double deionized water
4. drying at 60 °C
5. heating to 510 °C for 12 h
6. flushing twice with double deionized water

2.6.2 Degradation and diffusion of litter organic matter in soil ("source diffusion" experiment)

Degradation-diffusion experiments were conducted with ^{14}C-labeled biomass. ^{14}C labeled biomass was produced in two different ways: Plant biomass was produced by growing barley in an atmosphere containing ^{14}C-labeled CO_2 (see section 2.2). Microbial biomass was harvested after incubation of soil microorganisms in a medium with ^{14}C-labeled Glucose (see section 2.3).

The experiments were conducted in four replicates and with two different soils ("Cambisol 1" and "Arenosol", see section 2.1).

Polypropylene-tubes of diameter 29 mm were constructed from 60 mL syringes (item no. 22050, Dispomed Witt oHG, Germany). Preincubated (Ψ = -15 kPa) soil was packed in the tubes to a bulk density of 1.3 g cm^{-3}. Dried and ground ^{14}C-labeled biomass was mixed thoroughly with an oven dry (105 °C) aliquot of the same soil. This mixture was then mixed with preincubated soil to give a concentration of 0.02 g (barley) g^{-1} and 0.10 g (microbial biomass) g^{-1}, respectively. Subsequently it was wet to the desired water content (equivalent to Ψ = -15 kPa) and added as a 2 mm layer to one end of the soil column. The resulting total length of the soil column was 30 mm.

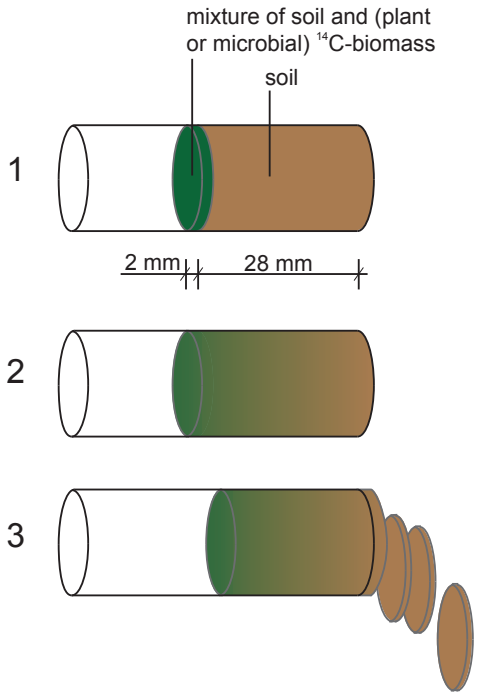


Figure 2.4: Principle of the "source diffusion" experiment. A 2 mm layer of ^{14}C-biomass-soil mixture is added to a 28 mm soil column (1). ^{14}C-labeled DOM diffuses into the column during incubation (2). In the end of the experiment the soil column is pushed out of the tube and cut into approximately 2 mm slices (3) which can be analyzed.

Immediately after packing the soil columns were transfered to a closed desiccator. The soil columns were placed over an expanse of water to prevent drying. At appropriate time points (daily in the beginning, twice a week in the end) the desiccator was aerated for 1 hour at a flow rate of 10 L h^{-1}. The air flowing in was humidified over 90 °C warm water, again to prevent drying. Air was sucked from the desiccator through two absorption tubes (Grundmann et al., 2007) containing a mixture of EDB to trap CO_2. The ab-

sorption liquid was sampled immediately and analyzed for ^{14}C radioactivity in a liquid scintillation counter (section 2.11.1).

After 28 days (one treatment 33 days) the soil columns were removed from the desiccator, pushed out of the tubes using a specially constructed device, and cut into approximately 2 mm slices. The weight of the columns as well as the weight of the slices was determined. An aliquot from each slice was pooled with aliquots from slices matching in position from the other replicates for determination of microbial biomass carbon (section 2.8). Water content was determined of three slices from each replicate, one from the middle, two close to the ends of the soil columns. Furthermore the samples were analyzed for total ^{14}C radioactivity in soil.

2.6.3 Diffusion of a pesticide as a result of degradation in microbial hot-spots ("sink diffusion" experiment)

A degradation-diffusion experiment was conducted with ^{14}C-labeled isoproturon (see section 2.4). The gradient driving diffusion was created by a microbial community which specifically degrades isoproturon and was placed in soil columns as a distinct hot-spot (Grundmann et al., 2007).

The experiment was conducted in 8 replicates with soil "Cambisol 3" (see section 2.1).

The specific isoproturon degrading microbial community was established (Grundmann et al., 2007) on expanded clay particles (Seramis, Masterfoods GmbH, Germany) by adding these to liquid culture in mineral salt medium (Sørensen et al., 2001) with ^{14}C-labeled isoproturon. The liquid cultures were aerated three times a week and CO_2 was trapped in absorption tubes containing 0.1 M NaOH solution. The NaOH solution was then measured for radioactivity in a liquid scintil-

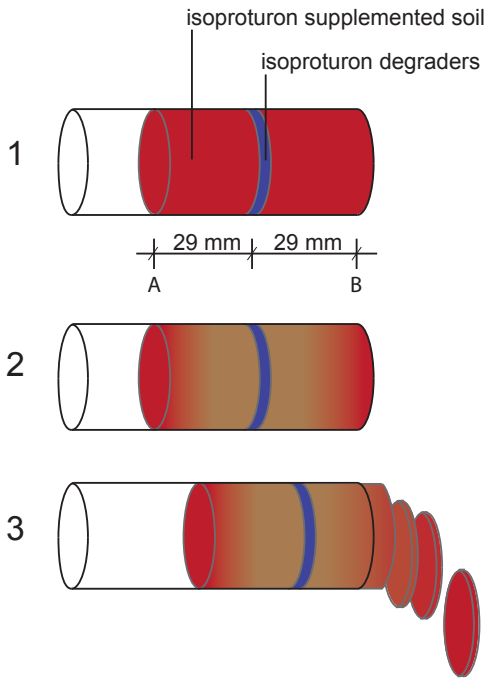


Figure 2.5: Principle of the "sink diffusion" experiment. A layer of isoproturon degraders is embedded in the middle of a column of isoproturon supplemented soil (1). Isoproturon is degraded and diffuses to the microbial degraders. As a result isoproturon concentration decreases towards the middle (2). In the end of the experiment the soil column is pushed out of the tube and cut into slices (3) which can be analyzed.

lation counter (see section 2.11.1). Shortly after the maximum mineralization rates had been reached (see appendix A, figure A.1), the expanded clay particles were taken out of the liquid cultures and immediately used to start the "sink diffusion" experiment. The microbial community had been isolated from a Calcaric Regosol (Grundmann et al., 2007).

Soil was preincubated one week at 12 % water content. 3.5 g (dry weight) aliquots of the soil were dried at 105 °C. Isoproturon was then applied to and mixed under the aliquots. Subsequently the aliquots were mixed with 51.5 g (dry weight) preincubated soil. Water was added to achieve a water content of 18.05 % corresponding to 15 kPa water tension (see figure 2.1). The final isoproturon concentration was 4.56 $\mu g\, g^{-1}$ dry soil.

PP-tubes as described in section 2.6.2 were used. First 2.9 cm soil were transfered in the tube and compacted to a density of 1.3 $g\, cm^{-3}$ in four steps. Then a layer of about 0.8 g expanded clay particles with the microbial community was added. Subsequently 2.9 cm soil were added again, so that the microbial habitats were situated in the middle of the soil columns. One column was prepared as a control sample without addition of the microbial community.

The soil columns were incubated in a desiccator and mineralization monitored as described in section 2.6.2.

After 43 days the soil columns were removed from the desiccator, pushed out of the tubes, and cut into approximately 2 mm slices. The weight of the columns as well as the weight of the slices was determined. An aliquot from each slice was pooled with aliquots from slices matching in position from three other replicates for determination of microbial biomass carbon (section 2.8). Water content was determined of three slices from each replicate, one from the middle, two close to the ends of the soil columns. The samples were then subjected to accelerated solvent extraction (ASE) (section 2.9.3) and the extracts were measured for radioactivity. Before analysis with high performance liquid chromatography (HPLC) (section 2.11.2) solid phase extraction (SPE) (section 2.9.3) was applied as clean-up. The remaining radioactivity in the extracted sample soil was determined after oxidation in a sample oxidizer (section 2.11.1).

Determination of apparent isoproturon diffusion coefficient in Cambisol 3

In a preexperiment (published in Grundmann et al., 2007) the apparent diffusion coefficient D_a of isoproturon in soil "Cambisol 3" at 15 kPa water tension was determined. The experiment was conducted in 6 replicates over 4 time periods.

The soil was preincubated 3 days at lab temperature with 0.1 % (mass) sodium azide at 12 % water content to inhibit microbial activity. Subsequently the soil was devided into two equal amounts and ^{14}C-labeled isoproturon was added to one subsample to give a concentration of 4.56 µg g^{-1} dry soil as described before. Water was added to the soil with and without isoproturon to achieve a water content of 18.05 % corresponding to 15 kPa water tension. The isoproturon supplemented and unsupplemented subsamples were then incubated at lab temperature for 2 days.

PP-tubes as described in section 2.6.2) were used again. 2.9 cm soil without isoproturon was filled in the tubes in four steps to a density of 1.3 g cm^{-3}. Subsequently 2.9 cm isoproturon supplemented soil was added in four steps to the same density. The soil columns were incubated in a desiccator over acidified water. An aliquot of soil with isoproturon and a beaker with 0.1 M NaOH was put in the desiccator to check for mineralization of isoproturon.

After 1, 2, 3, and 4 weeks the soil columns were removed from the desiccator, pushed out of the tubes, and cut into 2 mm slices. Water content was checked in three aliquots, two from close to the ends, one from the middle of each soil column. The samples were analyzed for total ^{14}C radioactivity in soil.

To calculate D_a the concentration profiles were fitted to the model (Gillham et al., 1984):

$$\frac{C}{C_0} = 0.5 + \frac{2}{\pi}\sum_{m=1}^{\infty}\frac{1}{m}\exp\left(\frac{-D_a m^2\pi^2 t}{l^2}\right)\cos\left(\frac{m\pi(l-x)}{l}\right)\sin\left(\frac{m\pi}{2}\right) \tag{2.5}$$

where C is concentration at time t, C_0 is starting concentration in soil with isoproturon, l is total length of soil columns, and x is position. Because the measured values were obtained by a counting process (see section 2.11.1) the variance structure of the data was assumed to be the variance structure of the Poisson distribution, i.e. the variance should be proportional to the number of counts. The χ^2 value of predicted versus measured values was taken as error and minimized by Levenberg-Marquardt-Method:

$$\chi^2 = \sum_{i=1}^{k}\frac{(c_i - E_i)^2}{c_i} \tag{2.6}$$

where k is number of values, c_i are measured values, and E_i are expected values.

2.7 Isoproturon sorption isotherm

Sorption of ^{14}C-labeled isoproturon on soil "Cambisol 3" was investigated in order to gain supplementary data for the "sink diffusion" experiment (section 2.6.3). A batch experiment was used to determine the isoproturon sorption isotherm (OECD, 2000). The experiment was done in quadruplicate. 4 g (dry weight) of soil was put into PTFE vials and 20 mL of an aqueous solution containing 0.1 % sodium azide and different concentrations (see table 2.5) of isoproturon were added. The vials were then put in an overhead tumbler for 72 hours. Subsequently the vials were centrifuged (4000×g, J2-21 centrifuge, Beckman Coulter, Inc., USA) for 20 min and the supernatend solution sampled and analyzed for radioactivity.

Table 2.5: Initial isoproturon concentrations for determination of sorption isotherm.

initial concentrations of isoproturon (mg L^{-1})					
0.3	1.5	7.5	15.0	22.5	30.0

The isoproturon equilibrium concentrations in solution and adsorbed on the soil matrix were calculated and subsequently a linear sorption isotherm ($q = K_d C$) and a Freundlich isotherm $\left(q = K_f C^{n_f}\right)$ were fitted to the data. Because standard deviations of concentration of sorbed pesticide were large compared to those of dissolved pesticide the error of dissolved concentration was neglected for regression. The variance structure of the data was considered as outlined in section 2.6.3.

2.8 Determination of microbial biomass

Microbial biomass in soil was assessed after experiments by measuring microbial carbon with the chloroform fumigation extraction method (DIN ISO 14240-2, 1999-10; Vance et al., 1987) modified for a lower soil : extractant ratio. Two aliquots were taken from each soil sample. One aliquot was extracted with 0.5 M K_2SO_4 solution at a soil : extractant ration of 1 : 8 (mass : mass) by shaking in an overhead tumbler for 45 min. The suspensions were filtrated (prewashed 595 1/2 filters, Whatman, Schleicher&Schuell) and frozen until analysis. The second aliquot was fumigated under chloroform atmosphere in a desiccator for 24 h and then treated like the nonfumigated aliquot. The extracts were analyzed for DOC (see section 2.10.1) and ^{14}C (see section 2.11.1). Microbial carbon C_{mic} was then calculated as:

$$C_{mic} = \frac{DOC_f - DOC_{nf}}{k_{EC}} \tag{2.7}$$

where DOC_f and DOC_{nf} are DOC concentration in extracts of fumigated and nonfumigated soil samples, and k_{EC} is a correction factor for extraction efficiency (Joergensen, 1996). Microbial ^{14}C concentration in soil was calculated in the same way. The influence of extraction ratio on k_{EC} was checked in a preexperiment. Although extracted DOC strongly depended on the extraction ratio, no significant differences between microbial carbon results from the standard extraction ratio of 1 : 5 and the adapted extraction ratio were detected (see appendix A, table A.1 for details).

2.9 Extraction procedures

2.9.1 Extraction of DOM from barley biomass

In order to obtain DOM from plant biomass for the "free diffusion" experiment barley plant material was extracted. The barley plants were grown for four weeks in an open pot with soil "Cambisol 1" after fertilization of the soil with Murashige&Skoog medium. Before extraction the dried (45°C) barley plants were ground in a ball mill (MM 2, Retsch, Germany). The ground barley plants consisted of 40.6 %(mass) carbon and 3.2 %(mass) nitrogen. 1 g of barley biomass was extracted with 10 mL 0.01 M $CaCl_2$ solution by shaking in an overhead shaker for 45 min. The suspension was filtrated through a 0.4 µm pore polycarbonate filter (Nucleopore, Whatman). For use in the "free diffusion" experiment the filtrate was diluted 1:50.

2.9.2 Extraction of soil solution by centrifugation

For extraction of soil solution from soil samples special centrifugation cups (Monreal and McGill, 1985; Nambu et al., 2005) were constructed. The cups consist of a middle part with a perforated bottom which holds the soil, a reservoir to collect the extract, and a lid. A 0.45 µm pore polyamide filter (NL17, Whatman) was placed over the perforations.

Undisturbed soil cores (80 cm^3, n = 20) from soils "Cambisol 1" and "Cambisol 2" were saturated to WHC with 10 mM $CaCl_2$ solution at 4 °C for 3 days in the dark. The soil cores were centrifuged for 72 hours at 4000 × g and 4 °C in a swinging bucket rotor (rotor: HS-4, centrifuge: Evolution RC, Sorvall, Thermo Electron). Water content was determined gravimetrically before and after centrifugation. Preliminary experiments showed that water content of the samples was nearly constant after 72 h of centrifugation (see appendix A, figure A.3 for details). All extracts were filtrated with a 0.4 µm pore polycarbonate filter (Nucleopore, Whatman) because

occasionally small amounts of soil bypassed the filter during centrifugation. Extracts were stored at -20 °C and analyzed for inorganic carbon (IC), DOC, and total nitrogen bound (TNb) (see appendix A, tables A.2 and A.3 for results). At the end extracts from each soil were pooled for use in the "free diffusion" experiment (see section 2.6.1).

2.9.3 Accelerated solvent extraction of isoproturon metabolites

Isoproturon and its metabolites were extracted from soil using the ASE technique (Richter et al., 1996, ASE 200, Dionex, Germany). Samples are enclosed in sample cartridges that are filled successively with methanol as extraction fluid. The samples are statically extracted under elevated temperature (90 °C) and pressure (10 MPa) for short time spans (each sample three times 5 min). Compressed nitrogen is used to purge the sample extract from the cell into a collection vessel. It has be shown by Schroll and Kühn (2004) that these conditions are suited for quantitative extraction without artifacts.

Clean-up of ASE extracts for HPLC

For analysis of ASE soil extracts with HPLC a clean-up (Schroll and Kühn, 2004) was necessary. The sample extracts were first concentrated to a volume of about 2 mL using rotary evaporators (Büchi Labortechnik, Germany). They were then diluted with double deionized water (Milli-Q Plus, Millipore) to a volume of 250 mL. Radioactivity was analyzed (section 2.11.1) in aliquots of the diluted extracts. The extracts were then passed through SPE columns with a non-polar retention mechanism (Bond Elut ENV 200 mg, Varian, Germany). The amount of radioactive substances not adsorbed to the SPE columns was determined. After drying the SPE columns in a soft nitrogen stream, they were eluted with 10 mL methanol. The extracts were analyzed for radioactivity, concentrated using rotary evaporators to a volume of 30 µL, and subsequently injected to an HPLC system (section 2.11.2).

2.10 Analysis of DOM

2.10.1 Analysis of dissolved organic carbon, inorganic carbon, and total nitrogen

Disolved carbon and bound nitrogen (all nitrogen excluding N_2) in extracts were measured using a TOC analyzer (DIMA-TOC 100, DIMATEC, Germany) with TNb module. The analyzer uses the following measuring principles: Total carbon (TC) is determined by thermal catalytic

oxidation (850 °C) of the sample and detection of CO_2 with a nondispersive infrared (NDIR) sensor. Nitrogen oxides in the exhaust gas of the TC detector are converted to NO with a catalyzer. In a reaction chamber ozone is added and occurring chemiluminescence is detected with a photomultiplier. For measurement of IC samples are passed through a hot (160 °C) phosphoric acid catalyzer and CO_2 is detected with a second NDIR sensor.

Table 2.6: Standard stock solutions for TOC/TNb analyzer

	TC	**IC**	**TNb**
concentration	1.000 g (carbon) L^{-1}	1.000 g (carbon) L^{-1}	1.000 g (nitrogen) L^{-1}
standard mixture	2.125 g L^{-1} potassium hydrogenphthalate	4.415 g L^{-1} Na_2CO_3 3.500 g L^{-1} $NaHCO_3$	3.610 g L^{-1} KNO_3 2.357 g L^{-1} $(NH_4)_2SO_4$

DOC of 0.4 µm filtrated samples was calculated as the difference of TC and IC. Each sample was measured three times. Samples could be analyzed reliably in a concentration range from 1 mg (carbon) L^{-1} up to 120 mg (carbon) L^{-1} for TC and IC and from 0.5 mg (nitrogen) L^{-1} up to 120 mg (nitrogen) L^{-1} for TNb. For samples in a matrix very low in salt content concentrations as low as 0.1 mg (carbon/nitrogen) L^{-1} could be measured with acceptable errors (± 0.02 mg (carbon/nitrogen) L^{-1}). Table 2.6 shows the standard stock solutions. For all standards double deionized water (Milli-Q Plus, Millipore) was used.

Extracts from the chloroform fumigation extraction method (see section 2.8) were measured using a Shimadzu TOC 5050 (Shimadzu, Japan) as this machine is more resistant to high salt concentrations in samples. Samples were acidified with 2 M HCl and IC removed by stripping the samples with oxygen 4.5 prior to measurement. The DOC was then measured as TC with the same principle as explained before.

2.10.2 Analysis of nitrogen species

A continuous flow analyzer (SA 20/40, Skalar Analytical, The Netherlands) was used to measure nitrate-N, ammonium-N, and TNb. Nitrate was reduced to nitrite and determined photometrically (540 nm, concentration range: 200 µg (nitrogen) L^{-1} - 5000 µg (nitrogen) L^{-1}). Ammonium was measured photometrically (660 nm) as indophenol after modified Berthelot reaction (concentration range: 25 µg (nitrogen) L^{-1} - 500 µg (nitrogen) L^{-1}). TNb was determined after oxidation by Griess reaction as nitrate. Dissolved organic nitrogen (DON) could then be calculated as the difference $DON = TNb - [NH_4^+ - N] - [NO_3^- - N]$ (concentration range: 200 µg (nitrogen) L^{-1} - 7000 µg (nitrogen) L^{-1}). Sodium nitrate, ammonium chloride, and the

Titriplex III standard provided by the manufacturer of the analyzer were used as standards (see table 2.7).

Table 2.7: Standard stock solutions for nitrogen analyzer

	nitrate	**ammonium**	**TNb**
concentration	1000 mg (nitrogen) L^{-1}	1000 mg (nitrogen) L^{-1}	1000 mg (nitrogen) L^{-1}
standard substances	6.071 g L^{-1} $NaNO_3$	3.818 g L^{-1} NH_4Cl	13.294 g L^{-1} Titriplex III

2.10.3 Assessment of DOM quality using excitation emission matrix fluorescence spectroscopy

Fluorescence spectroscopy is a convenient and therefore widely used tool for the assessment of quality, e. g., composition, of DOM (Ghosh and Schnitzer, 1980; Senesi, 1990; Senesi et al., 1991; McKnight et al., 2001). Fluorescence measurement is easy, quick and precise. In the last years excitation emission matrix fluorescence spectroscopy (EEMS) became the state-of-the-art technique for fluorescence measurements (Hudson et al., 2007) as a result of improved instruments and evaluation procedures. In principal, fluorescence intensity is scanned at a range of excitation and emission wave lengths. This results in an excitation emission matrix (EEM) which depicts intensity as a function of emission and excitation wavelengths. If visualized as a 3D plot such a "landscape" shows distinctive peaks which represent groups of fluorophores. Since these peaks are in the same areas as peaks from samples of known DOM fractions (e.g., humic acids, fulvic acids or some amino acids) they are interpreted as characteristic of these fractions. Semiquantitative and if standards are available even quantitative evaluation of EEMs is possible.

Because fluorescence is pH dependent (Laane, 1982) samples were acidified with 20 µL of 2 M HCl to achieve pH 2 as a standard condition. Absorption properties of the samples were measured with a photometer (Varian Cary 50 Bio) for wavelengths from 240 to 600 nm with an increment of 5 nm. If absorbance at 255 nm was above 0.1, samples were diluted to avoid pronounced absorbance and concentration effects on fluorescence (Zsolnay et al., 1999). Subsequently fluorescence intensity was measured in a fluorescence spectrometer (Varian Cary Eclipse). The following settings were used:

- excitation wavelength: start 240 nm, stop 450 nm, increment 5 nm
- emission wavelength: start 300 nm, stop 600 nm, increment 5 nm

- slit wide: excitation slit 10 nm, emission slit 20 nm
- PMT detector voltage: 600 V

Fluorescence data were corrected for inner-filter effect with absorption data as suggested by Lakowicz (1999):

$$I_{corr} = I_{obs} \cdot 10^{0.5(A_{exc}+A_{em})} \tag{2.8}$$

where I_{corr} and I_{obs} are corrected and uncorrected fluorescence intensities, and A_{exc} and A_{em} are the absorbance values at the excitation and emission wavelength of the fluorescence intensity value. The correction is based on the assumption that the average path length of excitation and emission light is 50 % of the cuvette width, respectively.

Evaluation of fluorescence spectra using PARAFAC

For further evaluation of the EEMs the parallel factor analysis (PARAFAC) model (Bro, 1998) was applied. The model allows the identification of fluorescence peaks and the quantification of fluorophores in a sample set. It is assumed that fluorescence intensity is a trilinear function of excitation wavelength, emission wavelength and concentration of a fluorophore. The intensity matrix is modeled as:

$$\mathbf{I} = a\mathbf{bc} \tag{2.9}$$

where a is the concentration loading, and $\mathbf{b}$ and $\mathbf{c}$ are excitation and emission loading vectors of a fluorophore. If multiple fluorophores or fluorophore groups (called factors in PARAFAC) contributed to an EEM their intensity matrices are summed up:

$$\mathbf{I} = \sum_{i=1}^{n} a_i \mathbf{b}_i \mathbf{c}_i \tag{2.10}$$

where n is number of factors. The number of factors which is best suited to explain the data is decided by the sum of squared errors and the core consistency. In simple terms core consistency is a measure that drops down in case of overfitting, so the model with the highest number of factors that still shows a good core consistency should be selected. The model and numerical solver algorithms have been implemented as the N-way toolbox (Andersson and Bro, 2000, http://www.models.kvl.dk/source/nwaytoolbox/index.asp) for MATLAB (The MathWorks, Inc., USA).

In preparation for the model Raman and Rayleigh scatter peaks were removed and interpolated (Bahram et al., 2006) from the EEMs. Nonnegativity constraints were applied to the PARAFAC model since fluorescence intensity is always positive.

2.11 ^{14}C analytics

2.11.1 Liquid scintillation counter

Liquid scintillation counters were used for measurement of ^{14}C radioactivity in liquid samples. Three counters (Perkin Elmer, Inc., USA) with identical characteristics were used:

- Packard Tri-Carb 2800TR
- Packard Tri-Carb 1900TR
- Wallac Winspectral 1414

Szintillation cocktails (Perkin Elmer, Inc., USA) used were:

- Hionic Fluor for EDB samples,
- Permafluor E+ for samples from the sample oxidizer (see section 2.11.1),
- Ultima Flo AF for NaOH samples,
- Ultima Gold XR for other organic solvents and aqueous samples.

To correct results for quenching customized quench curves were applied. Each sample was measured three times 5 min.

Sample oxidizer

A sample oxidizer (Packard Model 307, Perkin Elmer, Inc., USA) was used to prepare solid samples for measurement in a scintillation counter. Aliquots of about 300 mg were taken from samples with known water content into CombustCones (Perkin Elmer, Inc., USA). A few drops of highly concentrated sugar solution were added to optimize sample oxidation. The aliquots were then oxidized on a glowing Pt wire (1000 - 1300 °C) in oxygen flow. CO_2 was trapped in absorber liquid (Carbo-Sorb E, Perkin Elmer, Inc., USA) and a scintillation cocktail (Permafluor E+) was added. At the end the mixture was analyzed in a liquid scintillation counter (see section 2.11.1).

2.11.2 HPLC

Relative amounts of ^{14}C labeled isoproturon and its metabolites in ASE soil extracts after clean-up (see sections 2.9.3 and 2.9.3) were determined with HPLC. The HPLC system consists of a Merck Hitachi L-6200 Intelligent Pump (Merck, Germany), a Merck Hitachi L-4250 UV-VIS Detector (240 nm, Merck, Germany), and a Berthold LB 506 C-1 HPLC-radioactivity monitor (Berthold, Germany). A LiChrospher 100 RP18 column (5 µm, 250×4 mm, Merck, Germany) was used. The mobile phase was water and acetonitril at a flow rate of 1.0 mL min^{-1}. A linear gradient program as outlined in table 2.8 was applied. The standard mix consisted of unlabeled isoproturon (IPU), 1-[4-(2-hydroxypropan-2-yl)phenyl]-3-methylurea (2-OH-Mono), 1-[4-(propan-2-yl)phenyl]urea (Dides-IPU), 1-methyl-3-[4-(propan-2-yl)phenyl]urea (Monodes-IPU), and isopropylaniline.

Table 2.8: HPLC gradient program used for measurement of isoproturon metabolites.

time (min)	water (%)	acetonitril (%)
0.0	95	5
15.0	40	60
20.0	40	60
25.0	95	5
35.0	95	5

Spectra were evaluated with the RadioStar (version 4.6.0.0) software (Berthold, Germany).

2.12 Statistics

Nonlinear regression was done using Mathematica 5 (Wolfram Research, Inc., USA). PARAFAC was done in MATLAB 6 (The MathWorks, Inc., USA). Statistical tests, such as t-tests and ANOVA, were calculated in SPSS 13.0 (SPSS, Inc., USA).

Chapter 3

Results

3.1 Diffusion of DOM and inorganic nitrogen in free aqueous solution ("free diffusion" experiment)

3.1.1 Diffusion properties of carbon and nitrogen species

Composition and concentration of the various extracts (table 3.1) used in the "free diffusion" experiment were fairly different.

Table 3.1: Concentration of carbon and nitrogen species in extracts of barley (1 : 50 dilution) and soils "Cambisol 1" and "Cambisol 2".

		"Cambisol 1"	"Cambisol 2"	barley (1 : 50)
DOC	mg (carbon) L^{-1}	21.8	15.7	96.0
IC	mg (carbon) L^{-1}	10.3	< 1	< 1
ammonium	µg (nitrogen) L^{-1}	207	299	1047
nitrate	mg (nitrogen) L^{-1}	0.9	14.1	5.7
DON	mg (nitrogen) L^{-1}	1.5	3.0	11.6
DOC/TNb		8.4	0.9	5.2

DOC/TNb-ratio was highest for extract from soil **"Cambisol 1"**. The value is in very good agreement with the C/N-ratio (C/N = 8.2) of the solid soil material. Nitrogen was dominated by DON. This corresponds well with the soil being sampled after harvest and before fertilization. Carbonate concentration of the soil solution was relatively high which corresponds with the neutral soil pH.

Extract from soil **"Cambisol 2"** showed a very low DOC/TNb-ratio of 0.9 which is an order of magnitude lower than the value of the solid soil material (C/N = 9.3). As the soil had been sampled shortly after fertilization the extract showed a high nitrate content. DON was higher

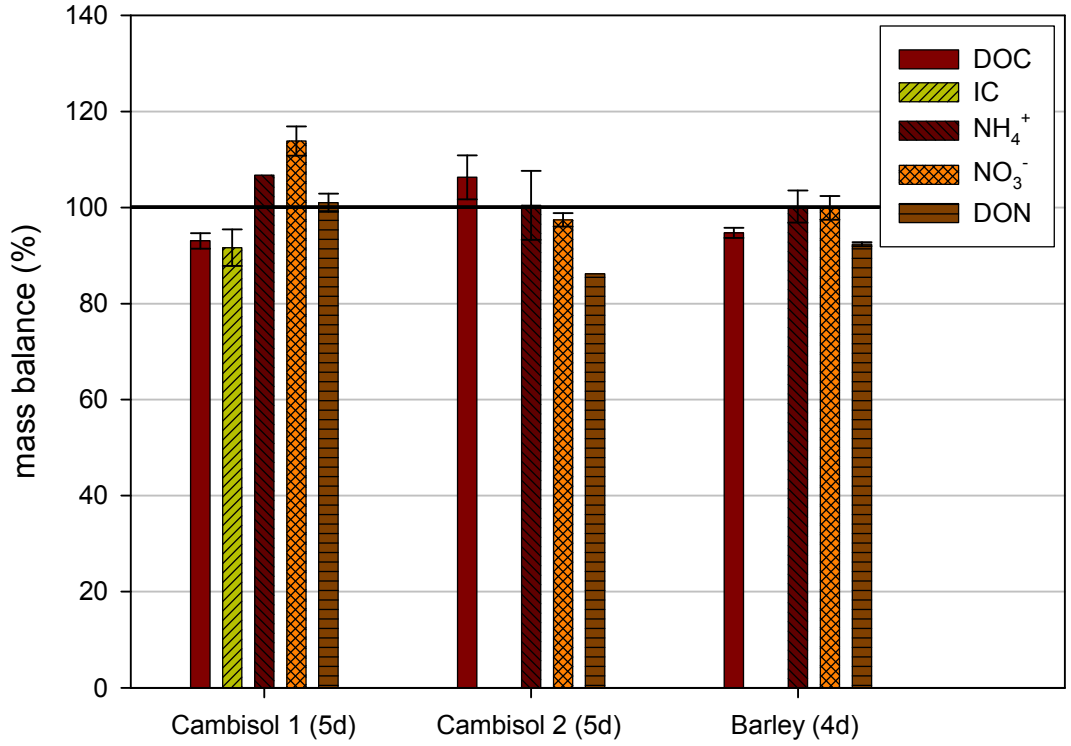


Figure 3.1: Mass balances of carbon and nitrogen species in the "free diffusion" experiment after five respectively four days. Samples were taken after four days for barley extract and after five days for soil extracts and compared to amounts applied. Values are based on replicates with sufficiently good mass balances (between 85 % and 115 %). For ammonium in soil "Cambisol 1" and DON in soil "Cambisol 2" only one replicate was included. All other values are based on three replicates or more. Error bars indicate standard deviation.

than in soil solution of soil "Cambisol 1". The extract contained nearly no carbonate which is in good agreement with the soil pH of 5.7.

DOC/TNb-ratio of **barley extract** was between the values of the soil solutions. It was lower than the C/N-ratio of the plants (C/N = 12.5) as expected because less soluble major components of plant biomass (like cellulose) contain no nitrogen. Nitrogen was mainly found in the form of DON.

Low concentration of a compound in the extracts frequently resulted in concentrations very close to detection limits in the receptor reservoir of the diffusion cells. This lead to some bad mass balances which were outside the preassigned range (see section 2.6.1) and the underlying values had to be discarded from the results. The most problematic compound was ammonium for which four out of five "Cambisol 1" replicates had to be discarded. DON was calculated from TNb and the inorganic nitrogen species. Because of accumulating errors this also lead

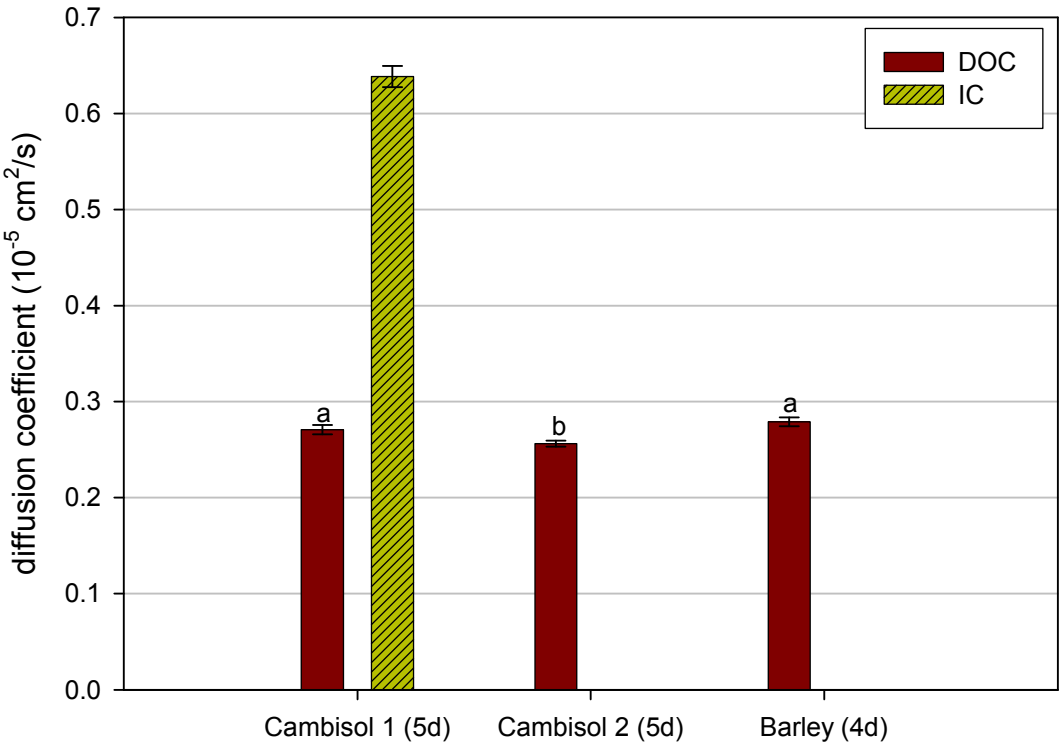


Figure 3.2: Diffusion coefficients of carbon species in aqueous solution at 4 °C. Values for DOC in extracts of barley and of soils "Cambisol 1" and "Cambisol 2" are given. In addition the value for IC in "Cambisol 1" extract is depicted. Samples were taken after four days for barley extract and after five days for soil extracts. Error bars show standard error of the mean. Values with the same letter assigned are not significantly different ($\alpha = 0.05$) according to One-way ANOVA and Tukey HSD test.

to some bad mass balances. Four out of five replicates were removed from "Cambisol 2" DON results. For all other compounds at least three replicates were available. The mass balances are depicted in figure 3.1. How much a mass balance differed from 100 % seems to depend mainly inversely on how far away measured values were from detection limit. There were no clear tendencies for balances of a compound to be always significantly below 100 %. Thus it is assumed that sorption to the frit of a diffusion cell was not a relevant problem.

Figure 3.2 shows diffusion coefficients of carbon in aqueous solution. Although the value for DOC originating from soil "Cambisol 2" was significantly lower than the values of the other samples differences were actually rather small. Inorganic carbon which is CO_3^{2-} and HCO_3^- exhibited a diffusion coefficient more than twice es high as the values for DOC. The value of $0.64 \cdot 10^{-5}\,cm^2$ is not significantly different ($\alpha = 0.05$) from the theoretical diffusion coefficient

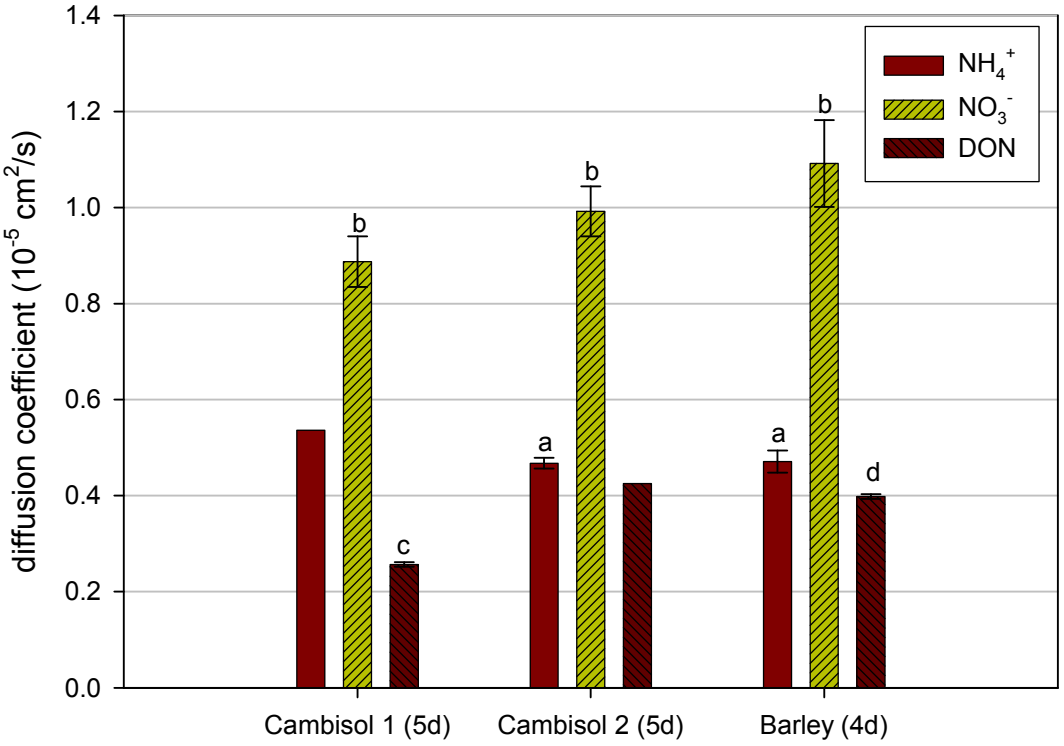


Figure 3.3: Diffusion coefficients of nitrogen species in aqueous solution at 4 °C. Values for ammonium, nitrate, and DON in extracts of barley and of soils "Cambisol 1" and "Cambisol 2" are given. Samples were taken after four days for barley extract and after five days for soil extracts. Error bars show standard error of the mean. Values with the same letter assigned are not significantly different ($\alpha = 0.05$) according to One-way ANOVA and Tukey HSD test. Because of very low concentrations only one replicate showed a sufficient mass balance for ammonium in soil "Cambisol 1" and DON in soil "Cambisol 2" All other values are based on three replicates or more.

of the HCO_3^- ion at 4 °C of $0.63 \cdot 10^{-5}\,cm^2\,s^{-1}$ (calculated from molar conductivity in Wedler, 2004). HCO_3^- is the dominant inorganic carbon species at the neutral pH of soil "Cambisol 1".

The nitrogen species with highest mobility (figure 3.3) was nitrate. There was no significant difference according to soil solution between nitrate diffusion coefficients and they were in good agreement with the theoretical value of $1.0 \cdot 10^{-5}\,cm^2\,s^{-1}$ (calculated from molar conductivity in Wedler, 2004) at 4 °C. Ammonium diffusion coefficients were again independent of origin of the extracts but significantly ($\alpha = 0.05$) and substantially lower than the theoretical value which is equal to the nitrate diffusion coefficient. DON from soil "Cambisol 1" diffused significantly ($\alpha = 0.05$) more slowly than DON from the other extracts. DON was the nitrogen species with the lowest diffusion coefficient although ammonium did not diffuse much faster. DON diffusion coefficient in "Cambisol 1" extract was similar to the DOC diffusion coefficient.

In the other extracts DON diffusion coefficient were considerably higher then DOC diffusion coefficients.

3.1.2 Fluorescence spectra and diffusion properties of fluorophores

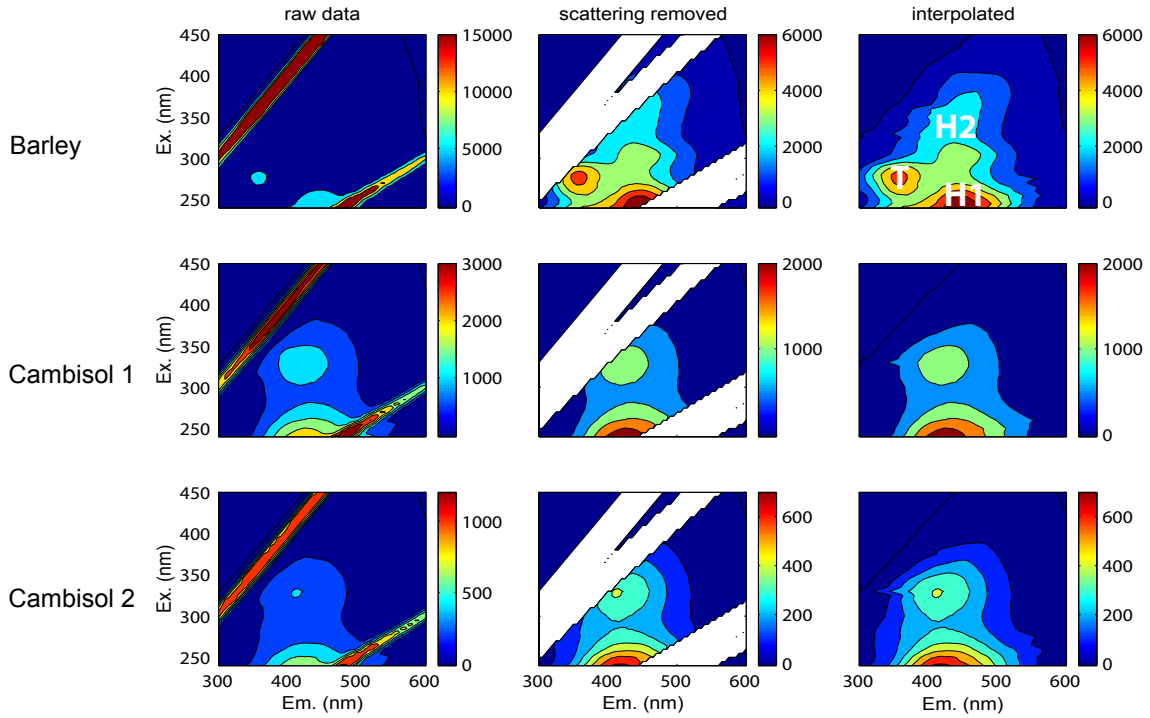


Figure 3.4: Fluorescence spectra of extracts from barley and soils "Cambisol 1" and "Cambisol 2". Spectra with scatter peaks, with scatter peaks removed, and with area of scatter peaks interpolated are shown. Three peaks (H1, H2, and T) can be distinguished optically.

All extracts showed distinct fluorescence properties (figure 3.4). In the soil extracts two peaks could be distinguished "by eye": One peak (H1) was in the region Emission: 380 - 500 / Excitation: 250 - 270 which is typical for humic and fulvic acid like substances (e.g., Chen et al., 2003; Hudson et al., 2007). A second peak (H2) was in the region Em.: 380 - 450 / Ex.: 300 - 350 which has been attributed to humic acid like substances (e.g., Chen et al., 2003; Hudson et al., 2007). Barley extracts exhibited a very clear third peak (T) in the region Em.: 320 - 380 / Ex.: 270 - 290. In this region substances usually attributed to microbial activity in soils, e.g., tryptophan and protein like compounds, show fluorescence (e.g., Chen et al., 2003; Hudson et al., 2007). Tryptophan is known to be abundant in barley shoots (Wightman et al., 1961).

Molecules can not emit more energy than was absorbed. Hence in the region of the EEM where excitation wavelengths are higher than emission wavelengths fluorescence is physically

impossible. The instrument returned small negative values in this region, which are clearly artificial. These values were not removed before modeling because handling missing values in the model requires substantial computation time. Applying non-negativity constraints to the model was sufficient to avoid an influence of the artificial values on the results .

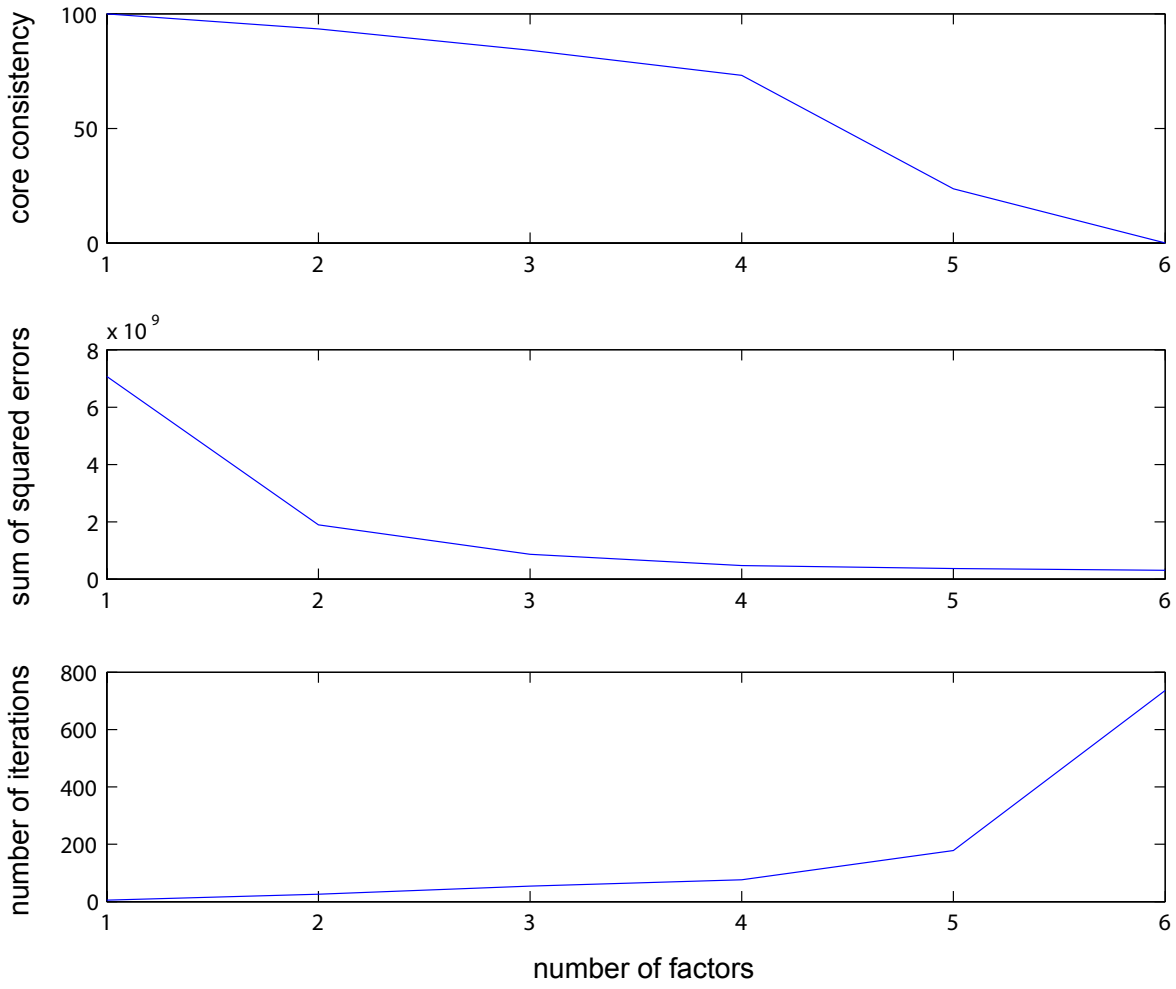


Figure 3.5: Quality parameters of the PARAFAC models as function of the number of factors. Core consistency, sum of squared errors, and number of iterations necessary to reach convergence are depicted. A rapid decline of model quality for more than 4 factors is apparent.

PARAFAC models were applied which took into account up to six factors. Figure 3.5 shows parameters which were used to find the number of factors best suited to explain the measured data. Core consistency slowly declined to about 75 % at 4 factors and than decreased sharply. According to the error of the model there was still some improvement from the three factor to the four factor model. The error stayed nearly constant when the number of factors was

increased above four. The number of iterations needed for the models to converge strongly increased for more than four factors. Taking into consideration all indicators and the plots of residues (e. g., figure 3.7) the four factor model was chosen.

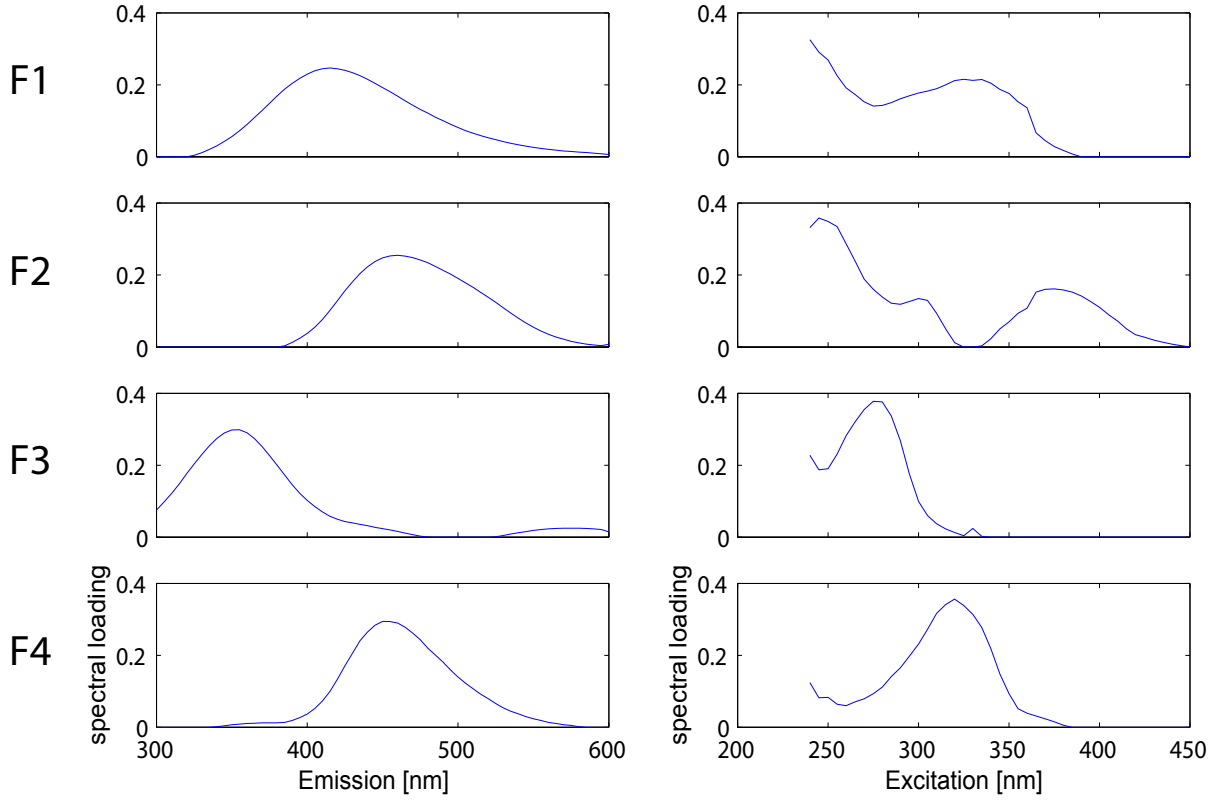


Figure 3.6: Spectral loadings of fluorophore groups as determined with PARAFAC for fluorescence data of all samples in the "free diffusion" experiment. Each line shows emission (left column) and excitation (right column) loadings of one of the four PARAFAC factors.

The spectral loadings of the four factors are depicted in figure 3.6. The product of emission and excitation loading vectors gives a matrix of intensities for one fluorophore with concentration 1. In general emission loadings show only one maximum, while excitation loadings show one or two maxima.

Factor 3 is very similar to the fluorescence signature of tryptophan (Stedmon and Markager, 2005). The other factors are typical of humic-like and fulvic-like substances (Stedmon and Markager, 2005; Ohno and Bro, 2006).

In case of fluorescence PARAFAC factors equal groups of fluorescent molecules or even single fluorescent substances. Since more than one fluorescent ring system may be part of a molecule, e.g., a humic substance (Hudson et al., 2007), some molecules show more than one

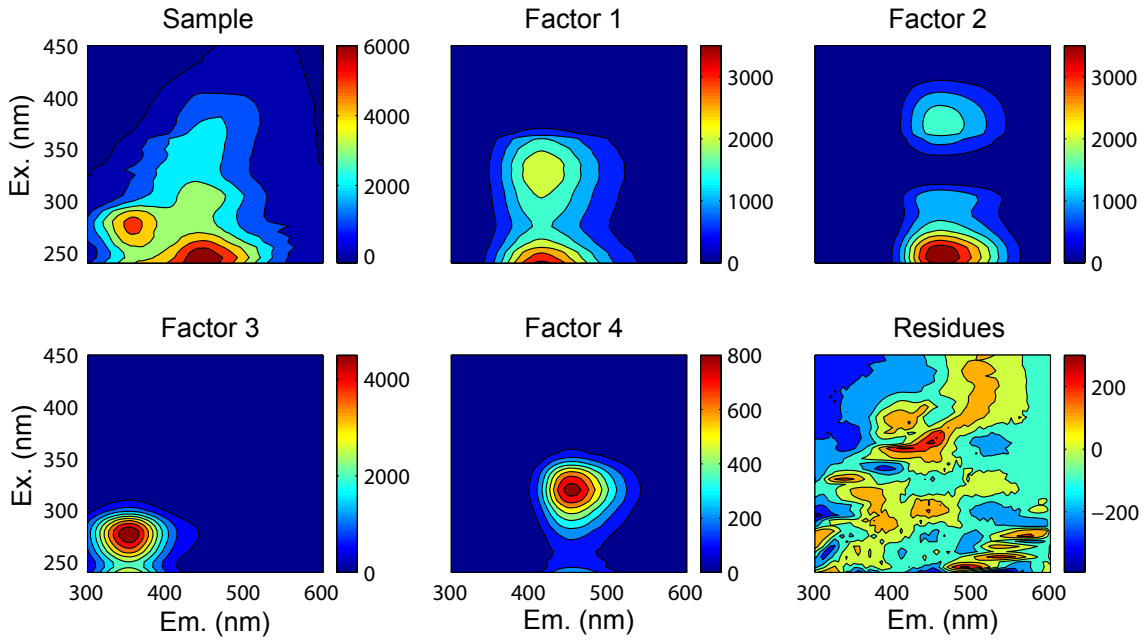


Figure 3.7: PARAFAC result for barley extract. The original spectrum, spectra of the fluorophore groups, and unexplained residues are shown.

fluorescence peak. Furthermore different substances can show peaks in overlapping or nearly identical positions which makes it often impossible to distinguish between them. The relatively wide peaks observed in this study, particularly for factors 1 and 2, hint that it is unlikely that they represent a single, well defined substance. However, for simplicity of language hereafter the PARAFAC factors are called fluorophores. For the above stated reasons it is not intended to imply by the use of this term that a PARAFAC factor represents a single molecular entity.

The PARAFAC model gives scores for the factors. Since the chemical structure of DOM is mostly undefined and standards are not available it is not possible to calibrate these scores to actual concentrations. However, it is possible to interpret the scores as concentrations (table 3.2) in arbitrary units and compare samples and fluorophores in a sample relative to each other.

Table 3.2: Concentrations (in arbitrary units per mg DOC) of fluorophores in extracts of barley and soils "Cambisol 1" and "Cambisol 2" as calculated by PARAFAC.

	"Cambisol 1"	"Cambisol 2"	barley
fluorophore 1	1065	474	482
fluorophore 2	427	157	443
fluorophore 3	87	89	395
fluorophore 4	9	0	76
sum	1588	720	1394

In the soil extracts concentrations decreased notedly from fluorophore 1 to fluorophore 4. Fluorophores 1 to 3 were present in all samples, but fluorophore 4 was not detected in soil DOM samples with low concentrations of DOC. Concentration of fluorophore 1 in "Cambisol 1" extract was more than double the concentration in "Cambisol 2" and barley extracts. Fluorophore 2 was equal in concentration in "Cambisol 1" and barley extract, while it was less abundant in "Cambisol 2" extract. Barley extract was characterized by a high concentration of fluorophore 3 compared to the soil extracts. The summed fluorescence per mg DOC in "Cambisol 2" extract was only about half of those in "Cambisol 1" and barley extracts.

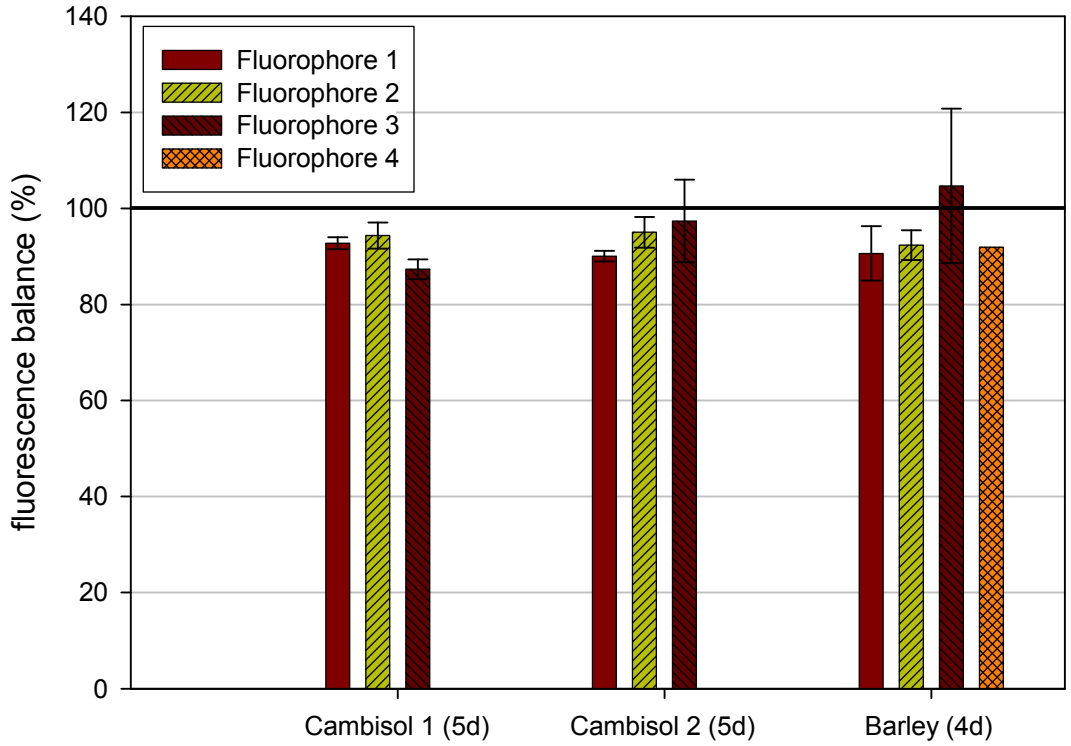


Figure 3.8: Fluorescence balances of the "free diffusion" experiment. Samples were taken after four days for barley extract and after five days for soil extracts. Values are based on replicates with sufficiently good fluorescence balances. Mean value for fluorophore 4 in experiments with barley extract is based on two values (96.7 and 87.2). All other values are based on three replicates or more. Error bars indicate standard deviation.

Figure 3.8 shows fluorescence balances of the fluorophores in the diffusion experiments. Again only values with sufficiently good balances, as defined in section 2.6.1, were included in the evaluation. Most problematic in this regard were fluorophores with low concentrations. Only two values of diffusion coefficients of fluorophore 4 met the criterion. This does not

allow a good estimate of mean value and standard error and fluorophore 4 was excluded from statistical analysis.

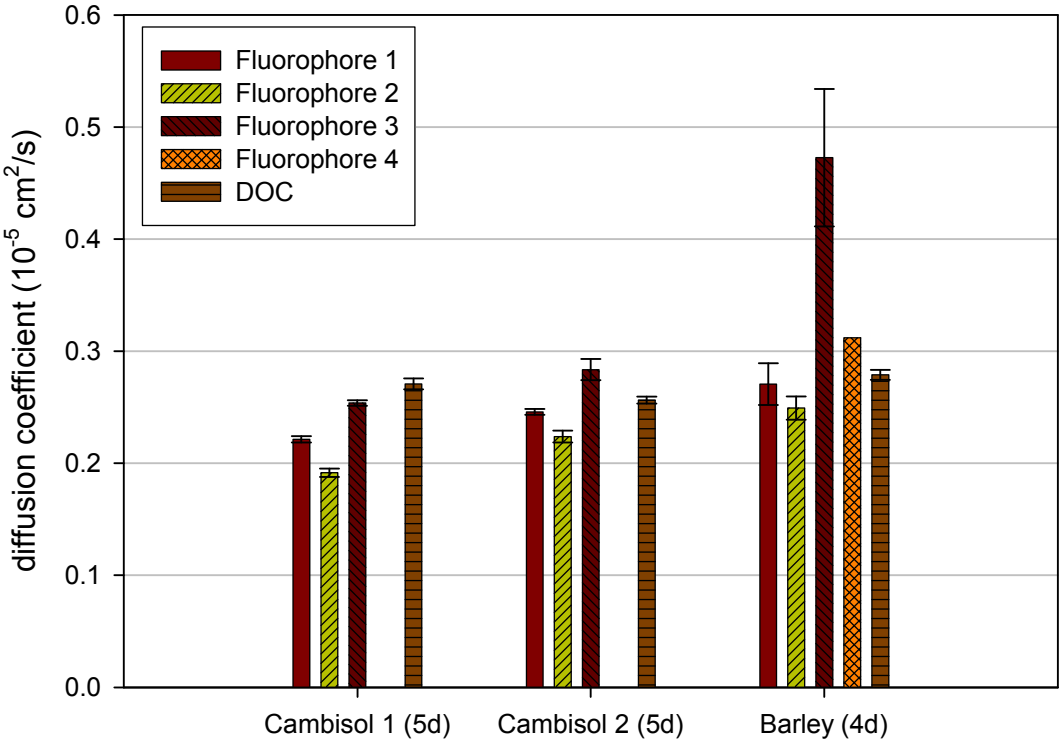


Figure 3.9: Diffusion coefficients of fluorophores in aqueous solution at 4 °C. Values for fluorophores in extracts of barley and of soils "Cambisol 1" and "Cambisol 2" are given. DOC diffusion coefficients were included. Samples were taken after four days for barley extract and after five days for soil extracts. Error bars show standard error of the mean. Mean value for fluorophore 4 in experiments with barley extract is based on two values (0.27 and 0.35). All other values are based on three replicates or more.

Diffusion coefficient of all fluorophores (figure 3.9) were quite similar among each other and to the diffusion coefficients of DOC. Only the value for fluorophore 3 in barley extract was substantially higher. For comparison the diffusion coefficient of tryptophan was calculated (Wilke and Chang, 1955) to $0.39 \cdot 10^{-5}\,cm^2\,s^{-1}$ (4°C). The molecular volume needed in the Wilke-Chang correlation was calculated using the molinspiration online calculator (http://www.molinspiration.com/cgi-bin/properties, Tetko, 2003).

Two-way ANOVA revealed significant ($\alpha = 0.05$) differences of diffusion coefficients according to origin of DOM and between fluorophores, and a significant interaction between these factors. Post-hoc tests (Tukey HSD, table 3.3) showed no significant differences between results for soils "Cambisol 1" and "Cambisol 2", but results for barley extract were significantly

Table 3.3: Results of Tukey HSD tests for fluorophore diffusion coefficients. Factors tested after two-way ANOVA were origin of DOM, and fluorophore / DOC. Significance level was $\alpha = 0.05$.

soil	"Cambisol 1"	"Cambisol 2"	barley	fluorophore	1	2	3	DOC
"Cambisol 1"			*	1			*	
"Cambisol 2"			*	2			*	*
barley	*	*		3	*	*		*
				DOC		*	*	

* significantly different

different to both. This difference is mainly caused by the high diffusion coefficient of fluorophore 3 in barley extract. Results for fluorophore 3 were significantly different to results for fluorophores 1 and 2. There were no further significant differences among fluorophores. Diffusion coefficients of fluorophores 2 and 3 were significantly different to diffusion coefficients of DOC which was not the case with fluorophore 1.

3.2 Degradation and diffusion of microbial and barley biomass in soil ("source diffusion" experiment)

3.2.1 ^{14}C-balance

Recovery of ^{14}C in the degradation-diffusion experiment with ^{14}C-barley biomass as a substrate was 106 % (soil "Cambisol 1") and 101 % (soil "Arenosol"). Results from experiments with ^{14}C labeled microbial biomass as a substrate showed strong variance in the ^{14}C content of the replicates after the experiment. The coefficient of variation for total ^{14}C amount in a replicate was 21 % in soil "Cambisol 1" and 10 % in soil "Arenosol". Because this was not the case with barley as a substrate (CV = 1 % for "Cambisol 1" and CV = 2 % for "Arenosol") it ist very unlikely that this is a result of different performance of the microbes in the replicates. It is assumed that this variation results from variation of the initial amount of substrate. Results showed that the samples of ^{14}C microbial biomass - soil mixture that were taken during the start of the experiment were not representative of the mixture applied to the soil collumns. Therefore no ^{14}C balance could be calculated for the experiments with ^{14}C labeled microbial biomass. Because of the identical setup and methods it is assumed that the balance would be in the same range as for the experiments with barley biomass as a substrate.

In all subexperiments of the "source diffusion" experiment results are given as percentage of applied ^{14}C or ^{14}C concentration. These values were calculated separately for each replicate from total amounts of ^{14}C measured after the experiment and from mineralization results.

3.2.2 Mineralization of ^{14}C-biomass in "source diffusion" experiment

Cumulative mineralization (figure 3.10) after 4 weeks was higher in soil "Cambisol 1" than in soil "Arenosol" with both substrates. The experiment with ^{14}C-microbial biomass in soil "Cambisol 1" was run slightly longer to check if mineralization rates would decrease and cumulative mineralization reach a plateau. This was not the case. In soil "Cambisol 1" both substrates were mineralized by about 50 %, whereas in soil "Arenosol" barley was mineralized by 46 % and microbial biomass by 40 % after 4 weeks. Curves for ^{14}C microbial biomass mineralization in both soils and barley mineralization in soil "Arenosol" exhibit a distinct point of inflection which concurs with the rate maximum. Mineralization before this point is likely limited by microbial activity. Mineralization after this point is limited by substrate availability. Mineralization of barley in soil "Cambisol 1" seems to never have been limited by microbial activity.

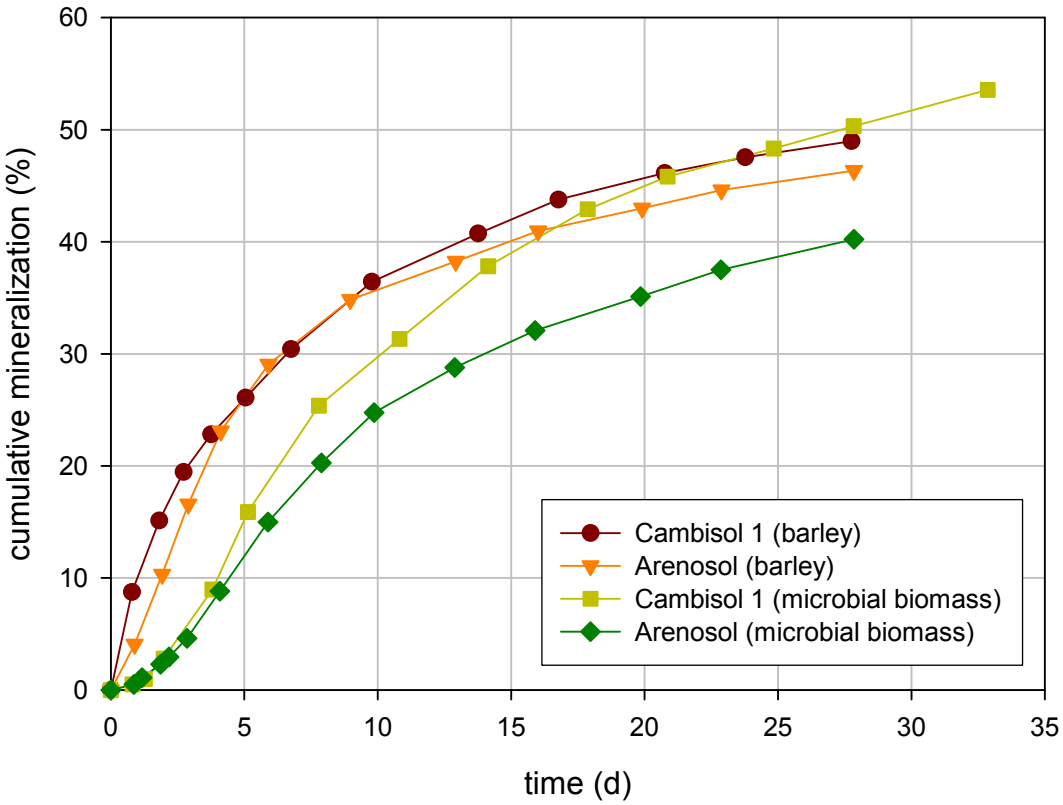


Figure 3.10: Mineralization of ^{14}C-barley litter and ^{14}C-microbial biomass in "source diffusion" experiment. Cumulative mineralization measured as ^{14}C-CO_2 released from soil is depicted as percent of applied ^{14}C for two soils and two substrates.

Mineralization rates (figure 3.11) for barley showed peaks very early in the experiment. In soil "Cambisol 1" the rate maximum was actually reached between application and the first aeration (19 hours). Rates then declined rapidly over the next 4 days, followed by a slow rate decline over the rest of the experiment duration. In soil "Arenosol" the rate maximum was only half of that in soil "Cambisol 1" and it was reached between 46 and 70 hours after application. The mineralization rates declined over the next 3 days and were then only slightly lower than those in soil "Cambisol 1".

Peaks of ^{14}C microbial biomass mineralization rates were later and lower, but wider than those for barley mineralization. The maximum was reached after about 5 days in both soils. Generally the rates in soil "Cambisol 1" were higher than those in soil "Arenosol".

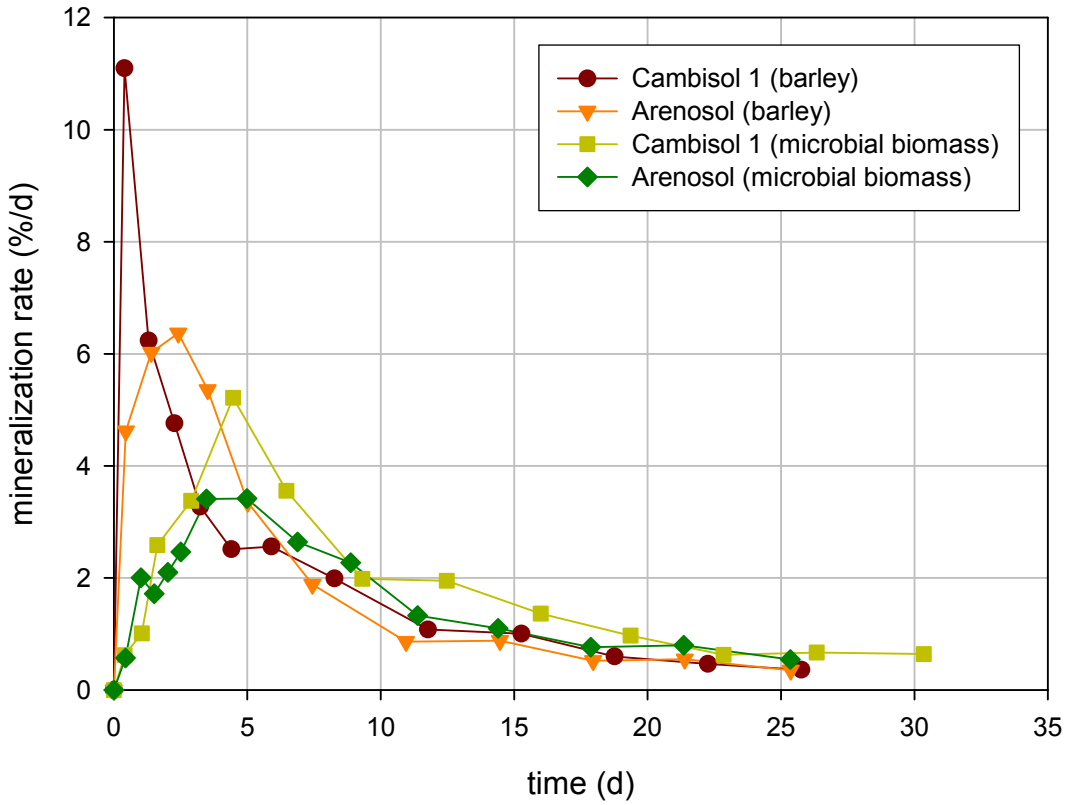


Figure 3.11: Mineralization rates calculated from cumulative mineralization of ^{14}C barley litter and ^{14}C microbial biomass in the "source diffusion" experiment are depicted as percent of applied ^{14}C mineralized per day.

3.2.3 Water content in soil after incubation

The soil columns were sliced and the experiment ended after 4 weeks (33 days for one treatment). Water content showed that a slight drying of the samples occurred during the experiment. Water contents at the end of the experiment are shown in table 3.4. Water content in the beginning of the experiment was 27.3 % and 10.1 % in soils "Cambisol 1" and "Arenosol", respectively. These water contents correspond to pF 2.18.

Although drying hampers quantitative evaluation of the results a bit, the results still allow investigation of the relevant processes. The reason for drying despite the supplied air being strongly humidified was possibly low pressure which might occur

Table 3.4: Water content after "source diffusion" experiments. Water tension at the start was pF 2.18 (27.3 % and 10.1 %, respectively).

"Cambisol 1"		"Arenosol"	
barley	**microbial biomass**	**barley**	**microbial biomass**
23.9 % ± 1.1 %	21.7 % ± 0.8 %	7.1 % ± 0.5 %	6.2 % ± 0.2 %
pF 2.57	pF 2.70	pF 2.89	pF 3.27

during aeration of the system. Low pressure means a lower partial pressure of water vapor which then can lead to drying.

3.2.4 Concentration profiles of total ^{14}C and ^{14}C in microbial biomass after the "source diffusion" experiment

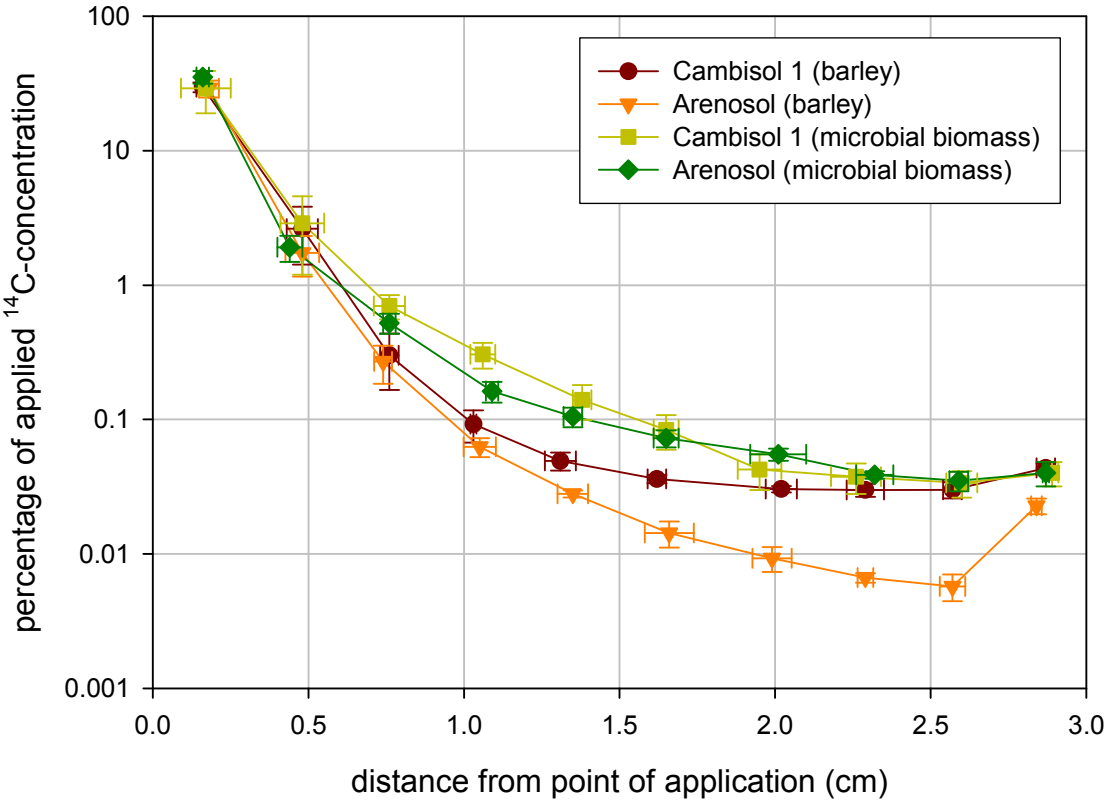


Figure 3.12: Total concentrations of ^{14}C plant litter and ^{14}C microbial biomass derived ^{14}C in the soil slices at the end of the "source diffusion" experiment. The concentrations are given on a logarithmic scale in % of applied ^{14}C concentration. Values are based on 4 replicates. Error bars indicate standard deviation.

Distribution of total ^{14}C concentration after the "source diffusion" experiment (figure 3.12) exhibits strong gradients close (within 0.5 cm) to source. Distinct gradients could be observed as far away as 1.5 cm from source. Beyond this distance measured radioactivity counts in soil columns with ^{14}C microbial biomass as substrate were mostly so close to detection limit that the values can be regarded as equal to zero. Generally detection limits for microbial biomass derived carbon were lower than those for barley derived carbon because of the higher radioactive label of the barley litter. About double the amount of radioactivity was applied to soil columns as barley litter then as microbial biomass substrate.

Mostly concentrations were higher in soil "Cambisol 1" than in soil "Arenosol". This indicates faster diffusion which can be expected from the higher water content of soil "Cambisol 1". Comparison of the two substrates shows that concentration in the soil column were higher for the ^{14}C microbial biomass substrate. The slight increase on the end of column without substrate is regarded as an experimental artifact. A possible reason could be fixation of ^{14}C-labeled CO_2.

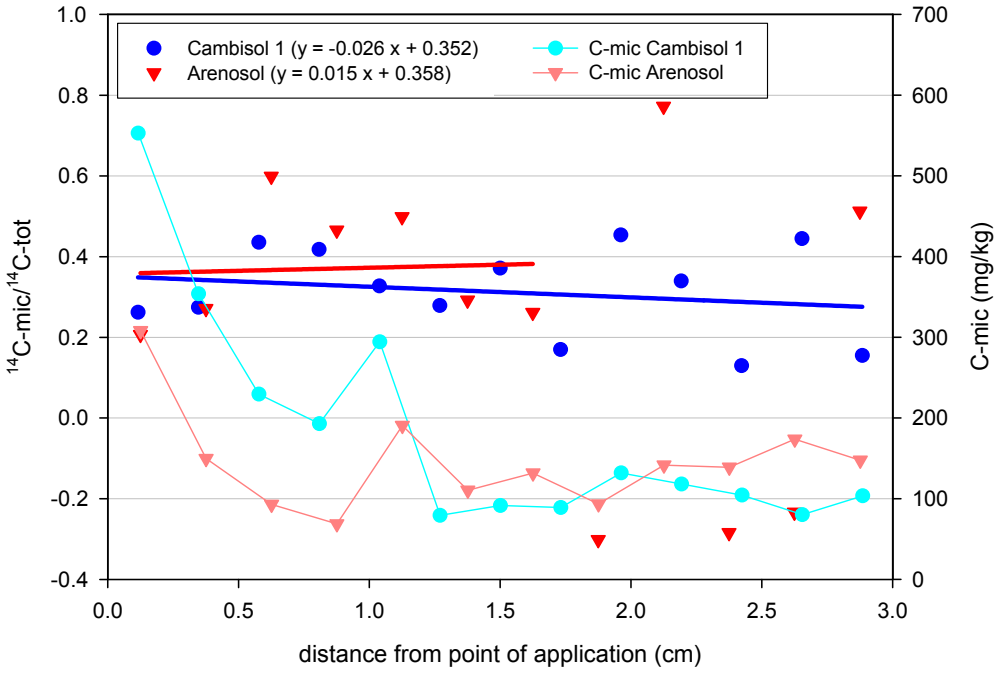


Figure 3.13: Fractions of ^{14}C-plant litter derived detected total ^{14}C in living microbial biomass and total amount of carbon in living microbial biomass (C-mic) in the slices at the end of the "source diffusion" experiment. Results are based on samples pooled from 4 replicates. Additionally results of linear regression are given. For soil Arenosol only part of the results was used in the regression due to extremely low radioactivity counts far from source.

Living microbial biomass carbon (C_{mic}) showed also distinct concentration profiles (figures 3.13 and 3.14). C_{mic} was strongly elevated at the point of substrate application and decreased to a constant level beyond 1 cm distance from point of application. C_{mic} at point of application was increased strongest in soil "Cambisol 1" by addition of barley litter. In soil "Arenosol" substrate effects were apparently not different.

Although there were high gradients of total ^{14}C-concentration the fraction of microbial ^{14}C ($^{14}C_{mic}$) was quite constant. The slope of linear regression lines was not significantly ($\alpha = 0.05$) different from zero. Because of extremely low concentrations of total ^{14}C as well es of microbial

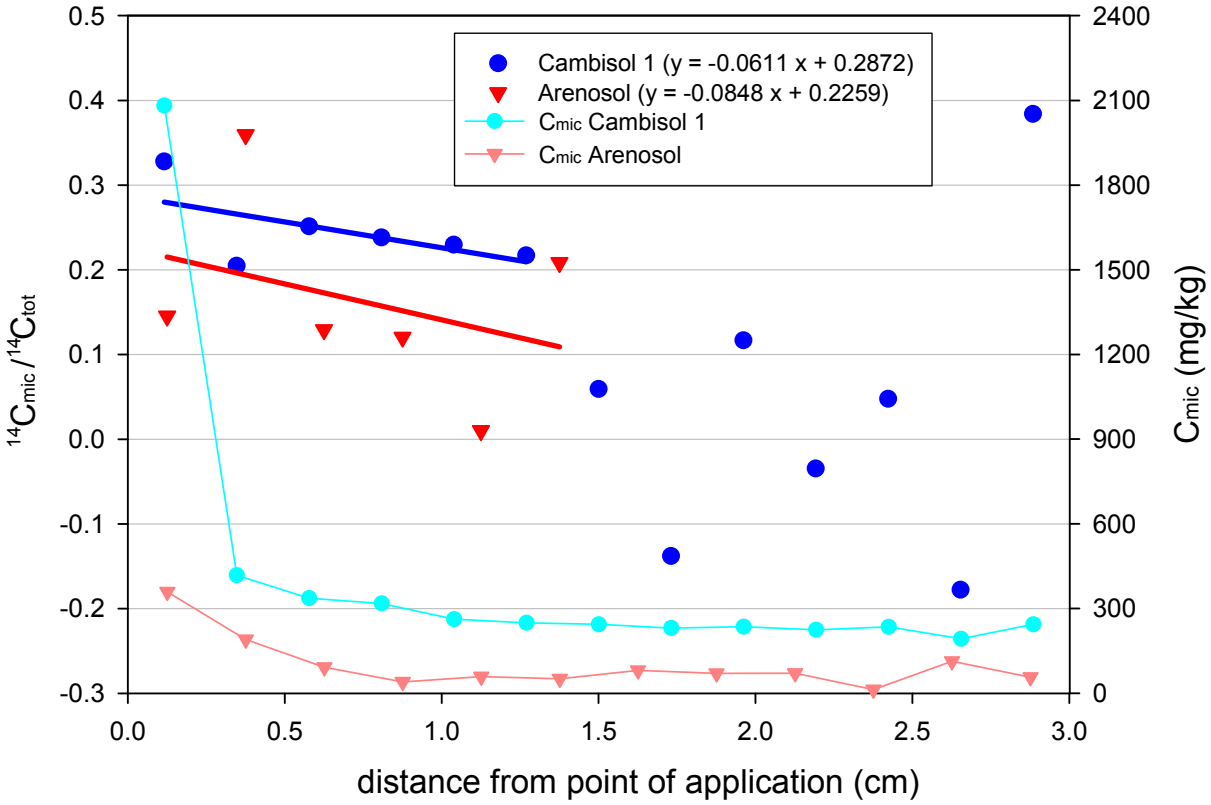


Figure 3.14: Fractions of ^{14}C-microbial biomass derived detected total ^{14}C in living microbial biomass and total amount of carbon in living microbial biomass (C-mic) in the slices at the end of the "source diffusion" experiment. Results are based on samples pooled from 4 replicates. Additionally results of linear regression are given. Only part of the ^{14}C-results was used in the regression due to extremely low radioactivity counts far from source. Part of the ^{14}C-results far from source for soil "Arenosol" were excluded from the graph to avoid a large scale.

^{14}C, errors mostly were unacceptably large for samples of the half of soil columns more distal to the point of application. Therefore only values from samples closer to the point of application were subjected to further statistics. Median values of $^{14}C_{mic}/^{14}C_{tot}$ were 0.33 (soil "Cambisol 1") and 0.29 (soil "Arenosol") in experiments with barley litter, and 0.25 (soil "Cambisol 1") and 0.14 (soil "Arenosol") in experiments with ^{14}C microbial biomass as a substrate. A two-way ANOVA was conducted on the data transformed into ranks. There was a significant ($\alpha = 0.05$) effect of the substrate, but no significant effect of soil and no significant effect of the interaction term on $^{14}C_{mic}/^{14}C_{tot}$. A two-away ANOVA on the untransformed data yielded the same results.

3.3 Diffusion of a pesticide as a result of degradation in microbial hot-spots ("sink diffusion" experiment)

3.3.1 Sorption of isoproturon on soil "Cambisol 3"

Sorption data (figure 3.15) of isoproturon on soil "Cambisol 3" could be explained very well by the linear sorption model ($q = K_d C$) with $K_d = 0.5487\,\mathrm{L\,kg^{-1}}$. The exponent of the Freundlich isotherm is close to 1. Furthermore model quality parameters such as asymptotic correlation matrix and curvature table indicate that the non-linear Freundlich model is not necessary to explain the measured data and the data are not sufficient for a good estimate of the Freundlich parameters. As a consequence sorption of isoproturon on soil "Cambisol 3" is considered to be linear for dissolved isoproturon concentrations below $27\,\mathrm{mg\,L^{-1}}$. Concentrations in soil during the "sink diffusion" experiment were calculated to be below this value.

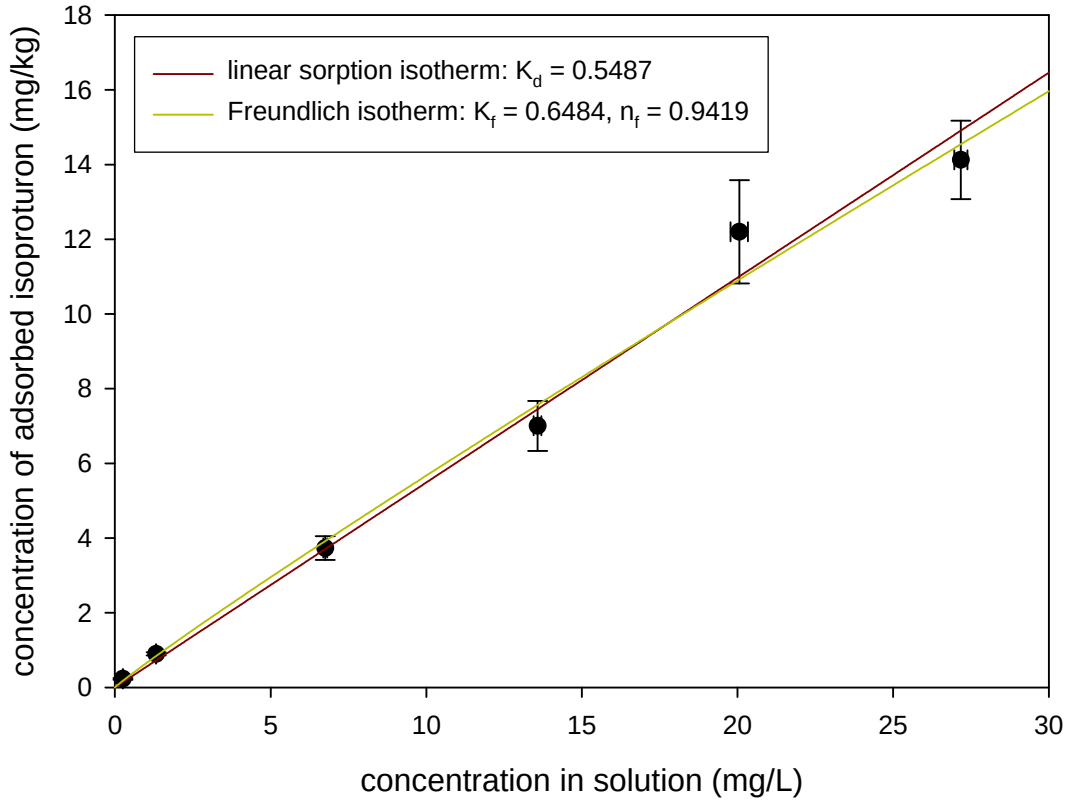


Figure 3.15: Equilibrium concentrations after adsorption of isoproturon on soil "Cambisol 3". Mean values of concentration of adsorbed pesticide are depicted versus concentration in solution. Error bars show standard deviation, mean values are based on 4 replicates. Additionally fitted linear sorption isotherm and Freundlich isotherm are given.

3.3.2 Apparent diffusion coefficient of isoproturon

The apparent diffusion coefficient of isoproturon in soil "Cambisol 3" seems to be slightly time dependent (figure 3.16). ANOVA followed by Tukey HSD test revealed a significant ($\alpha = 0.05$) difference only between values after one and four weeks. Because of the small differences it was decided to neglect the time dependency and take the overall median of $2.58 \cdot 10^{-7}$ cm^2 s^{-1} as the result. A time independent (and therefore concentration independent) apparent diffusion coefficient is in good agreement with linear sorption properties of isoproturon on soil "Cambisol 3" (section 3.3.1). Radioactivity balances of the replicates were 98.1 % ± 2.1 %. Values of two replicates at time point 3 weeks were discarded because of unsatisfactory balances. No ^{14}C-labeled CO_2 was released from soil during the experiment.

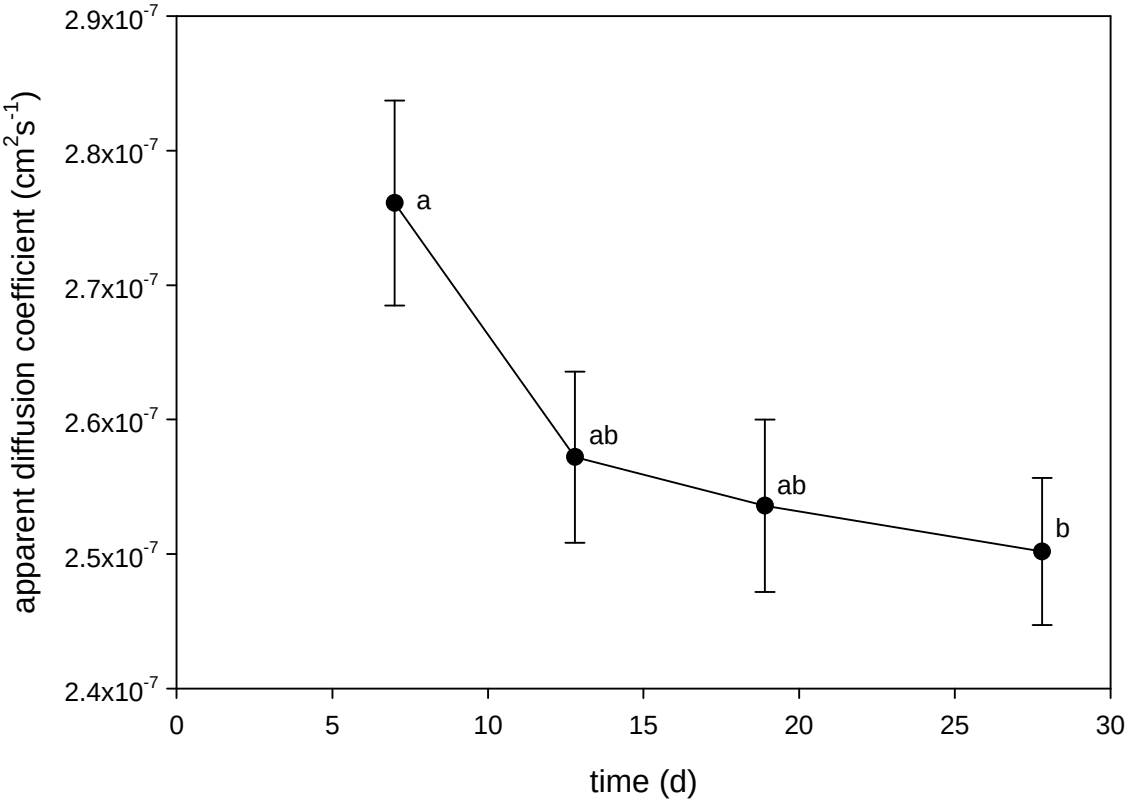


Figure 3.16: Apparent diffusion coefficient of isoproturon in soil "Cambisol 3" versus time. Note the scale of the axis of ordinate. Error bars show standard error. Mean value at time point 3 weeks is based on 4 replicates, all others on 6 replicates. Different letters indicate significantly ($\alpha = 0.05$) different values.

For comparison the diffusion coefficient was approximately calculated (see appendix B) using equation 1.6 and Archie's law (Archie, 1942):

$$D_e = D_{aq}\varepsilon^m \tag{3.1}$$

where ε is porosity and m is an empirical parameter close to 2 in soils (Grathwohl, 1998). The diffusion coefficient of isoproturon in water was calculated (Wilke and Chang, 1955) to $6.0 \cdot 10^{-6}$ cm^2 s^{-1}. The molecular volume needed in the Wilke-Chang correlation was calculated using the molinspiration online calculator (http://www.molinspiration.com, Tetko, 2003). The resulting apparent diffusion coefficient is $2.8 \cdot 10^{-7}$ cm^2 s^{-1} which is in excellent agreement with the experimentally determined value.

3.3.3 ^{14}C-balance of the "sink diffusion" experiment

In the experiment 95.9 % of applied ^{14}C were recovered considering ^{14}C-CO_2 and ^{14}C in all soil slices.

3.3.4 Mineralization of isoproturon

Mineralization of ^{14}C during the "sink diffusion" experiment, detected as evolving ^{14}C-CO_2, is shown in figure 3.17. At the beginning of the experiment mineralization rates increased rapidly to a maximum of about 1 % d^{-1}. Later they declined slowly to about 0.5 % d^{-1} after 27 days and stayed constant for about 3 days. Subsequently a sharp decline of the rates to 0.25 % d^{-1} occured and the rates stayed constant until the end of the experiment. The cumulative mineralization after 42.8 days was 24.85 %.

Figure 3.17 includes predictions from a simple diffusion model. The model assumes that mineralization is purely limited by diffusion, i.e. mineralization in the applied microbial hotspots is assumed to be instantaneous and mineralization by native soil microorganisms is neglected. The diffusion coefficient as determined in the preexperiment (section 3.3.2) was used to calculate diffusion. It is obvious that the model strongly overestimates mineralization rates in the first week of the experiment. This shows that mineralization was not limited by transport at the beginning, but by microbial activity. Although the isoproturon degrading community was transfered into soil on protected habitats, the expanded clay particles, the transfer seems to somewhat decrease the initial activity. From about day 15 to day 21 mineralization rates

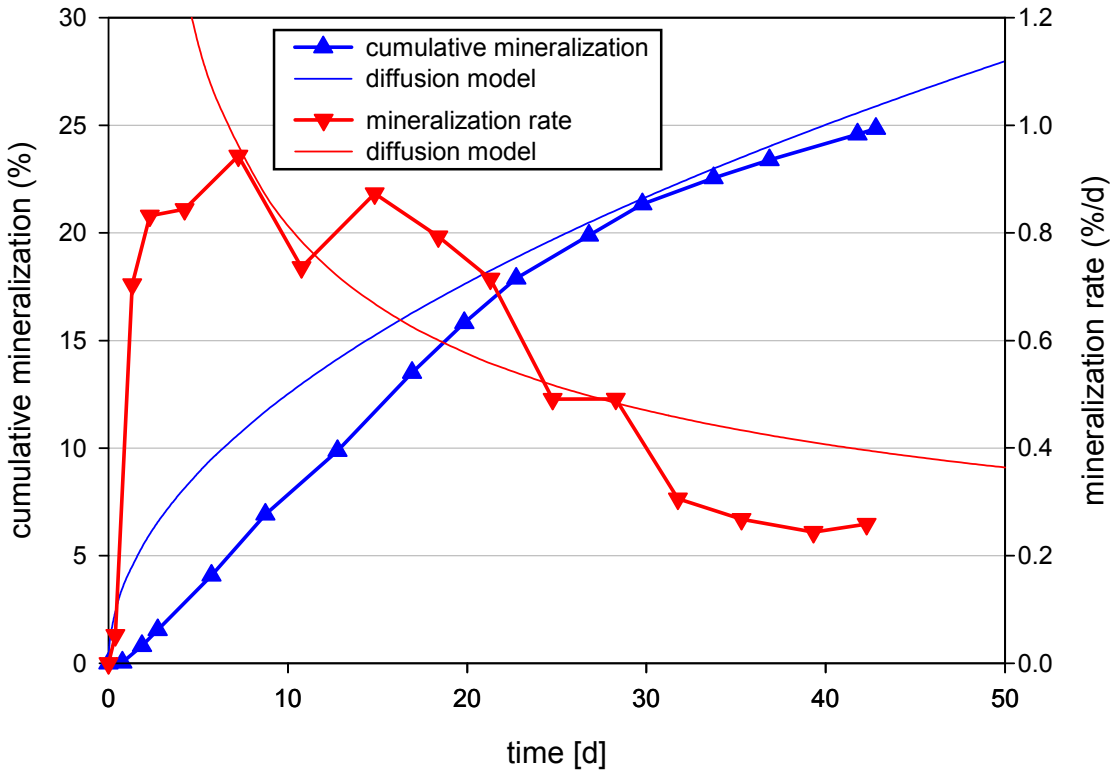


Figure 3.17: Mineralization of isoproturon in the "sink diffusion" experiment. Percent of applied ^{14}C released as CO_2 is depicted as cumulative mineralization and as mineralization rate calculated from cumulative mineralization. Additionally results of a diffusion model which considers maximum possible diffusion rates towards the microbial hot-spots are given.

were higher than predicted. After one month the rates were somewhat less then predicted. Overall cumulative mineralization was close to predicted values in the longer term.

3.3.5 Water content in soil after incubation

The soil columns were sliced and the experiment ended after 43 days. Water content showed that some drying of the samples occurred during the experiment. Water content (in $g\,g^{-1}$ dry substance) was 0.074 ± 0.036 (pF 3.65) close to the end A (see figure 2.5), 0.081 ± 0.032 (pF 3.62) close to the middle, and 0.080 ± 0.033 (pF 3.62) close to end B of the columns. The end A is defined here as the end where slicing was started and its measured values are depicted left in the graphs. Water content in the beginning of the experiment was 0.173 (pF 2.18). Although drying makes quantitative evaluation of the results difficult, the results still allow investigation of the relevant processes. The reason for drying despite the supplied air being strongly humidified was possibly low pressure which might occur during aeration of the system. Low pressure means a lower partial pressure of water vapor which then can lead to drying. The drop of the mineralization rates after about 30 days hints that drying occurred during this sampling.

3.3.6 Concentration profiles of isoproturon derived ^{14}C-fractions in soil

Total ^{14}C-concentration (figure 3.18) distributed symmetrically with a distinct "w"-like shape. Concentration decreased from the ends towards the microbial hot-spots and reached a minimum of less than 60 % of applied ^{14}C about 0.5 cm away from the middle. In the soil in close proximity to the microbial hot-spots concentrations of about 5 to 10 % above the minimum were observed. Total ^{14}C-concentration in the expanded clay particles was 172.7 % ± 28.7 %. The values at both ends of the soil columns are regarded as experimental artifacts.

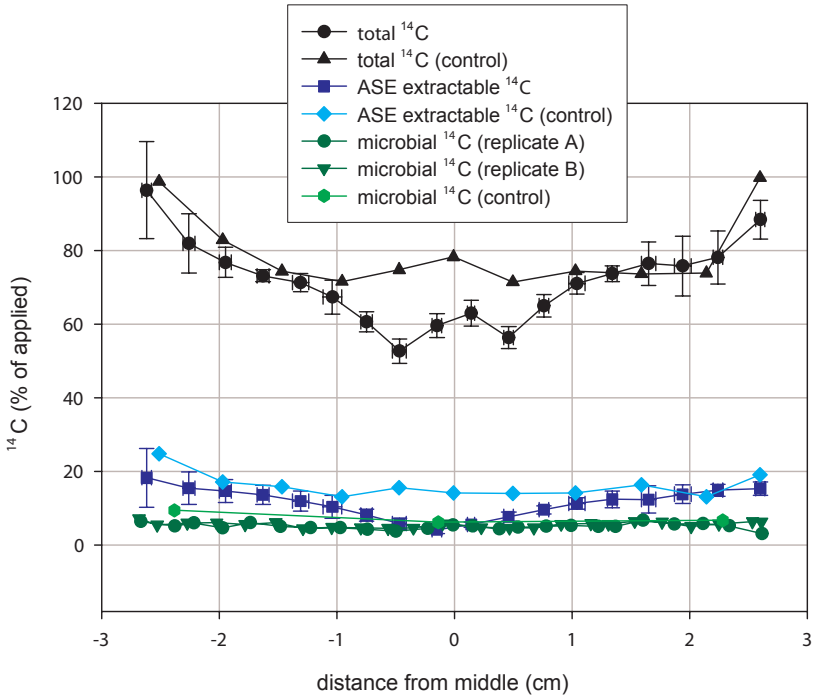


Figure 3.18: Concentration profiles for total ^{14}C, ASE extractable ^{14}C, and microbial ^{14}C in soil at the end of the isoproturon "sink diffusion" experiment. Values of total ^{14}C and ASE extractable ^{14}C are based on 8 replicates, the corresponding control values on one replicate and two adjacent slices. Values of microbial ^{14}C were determined from samples pooled from three replicates resulting in two pooled replicates (A and B). For the corresponding control values three adjacent slices were pooled. Error bars indicate standard deviation.

Microbial ^{14}C-concentrations ranged from 4 % to 7 % of applied ^{14}C. Although microbial ^{14}C distribution was fairly even, a closer look (figure: 3.19) reveals again a "w"-shape. Microbial ^{14}C in the expanded clay particles was with 10.8 % significantly higher than in soil. C_{mic} values (mg (carbon) kg^{-1} (dry soil)) were 76 ± 26 (replicate A), 115 ± 26 (replicate B), and 81 ± 32 (control). They were (within the accuracy of the method) constant and not correlated with position in the soil columns.

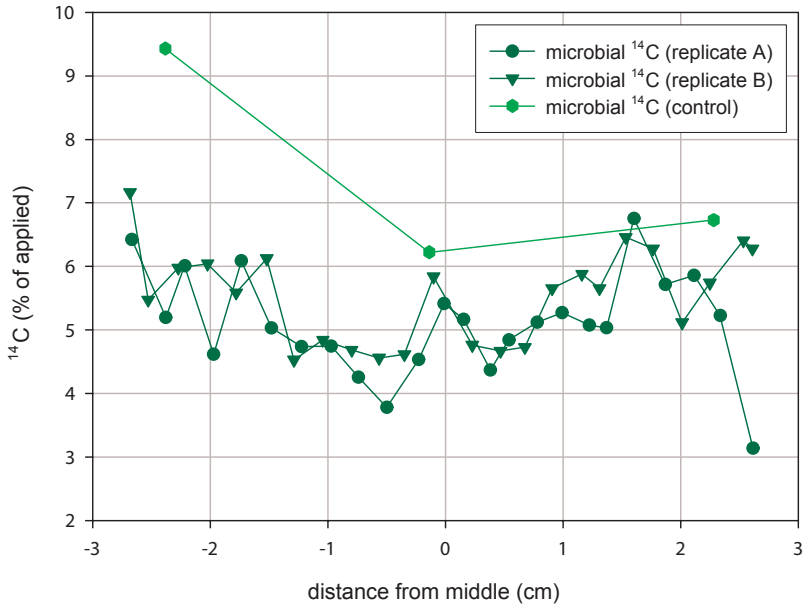


Figure 3.19: ^{14}C in microbial biomass in soil at the end of the "sink diffusion" experiment. Values of microbial ^{14}C were determined from samples pooled from three replicates. For the corresponding control values three adjacent slices were pooled.

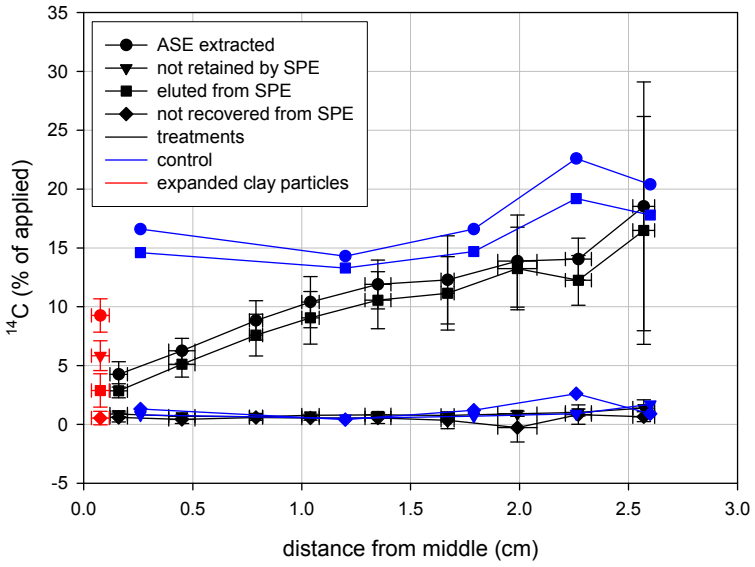


Figure 3.20: SPE clean-up in the "sink diffusion" experiment. Values of ^{14}C ASE extracted, not retained by SPE, eluted from SPE columns, and not recovered from SPE columns are given for treatments, control and expanded clay particles. The values are depicted versus distance from the middle of the diffusion tubes where the layer of expanded clay particles was located. Values for treatments are means of four samples **pooled from the two sides** of the concentration profiles of two experimental replicates. Error bars indicate standard deviation.

A very high proportion of total ^{14}C could not be extracted by ASE. Less than 20 % of applied ^{14}C were ASE extractable after the experiment. Extractable ^{14}C decreased significantly towards the middle of the soil columns and followed a concentration profile typical of diffusion.

In SPE cleanup (figure 3.20) the fractions of ASE extracted ^{14}C which could not be retained by the SPE columns or could not be eluted from the columns by methanol were quite low and constant throughout the soil columns. Since the SPE columns work with a non-polar retention mechanism, highly polar substances pass through and highly non-polar substances can not be eluted with methanol. Extracts from expanded clay particles contained a high proportion (about 63 % of extracted ^{14}C) of unretainable (polar) substances.

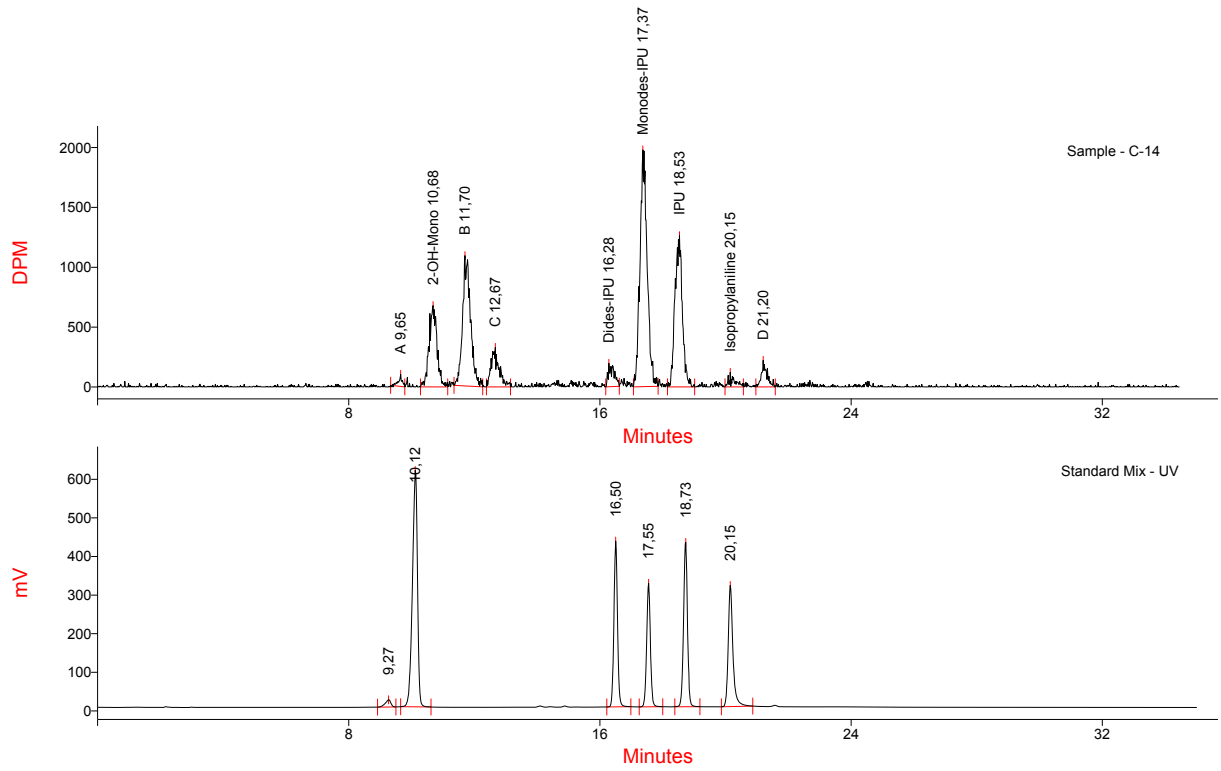


Figure 3.21: Example of an HPLC chromatogram of a sample from the "sink diffusion" experiment. The upper part shows the ^{14}C-peaks of the sample with retention times and peak names. The lower part shows the UV-chromatogram of the standard mix. The first peak in the latter is an unknown impurity.

HPLC measurements allowed to distinguish 9 different compounds (see figure 3.21 for an example chromatogram) in the ASE extracts. Standards were available for 5 compounds, so 4 compounds could not be identified. A standard HPLC chromatogram published by Schülein (1998) shows peaks in similar position relative to the known compounds as compounds A, B and C. These compounds are possibly 1-[4-(2-hydroxypropan-2-yl)phenyl]urea (2-hydroxy-didesmethyl-isoproturon, peak A), 3-[4-(2-hydroxypropan-2-yl)phenyl]-1,1-dimethylurea (2-

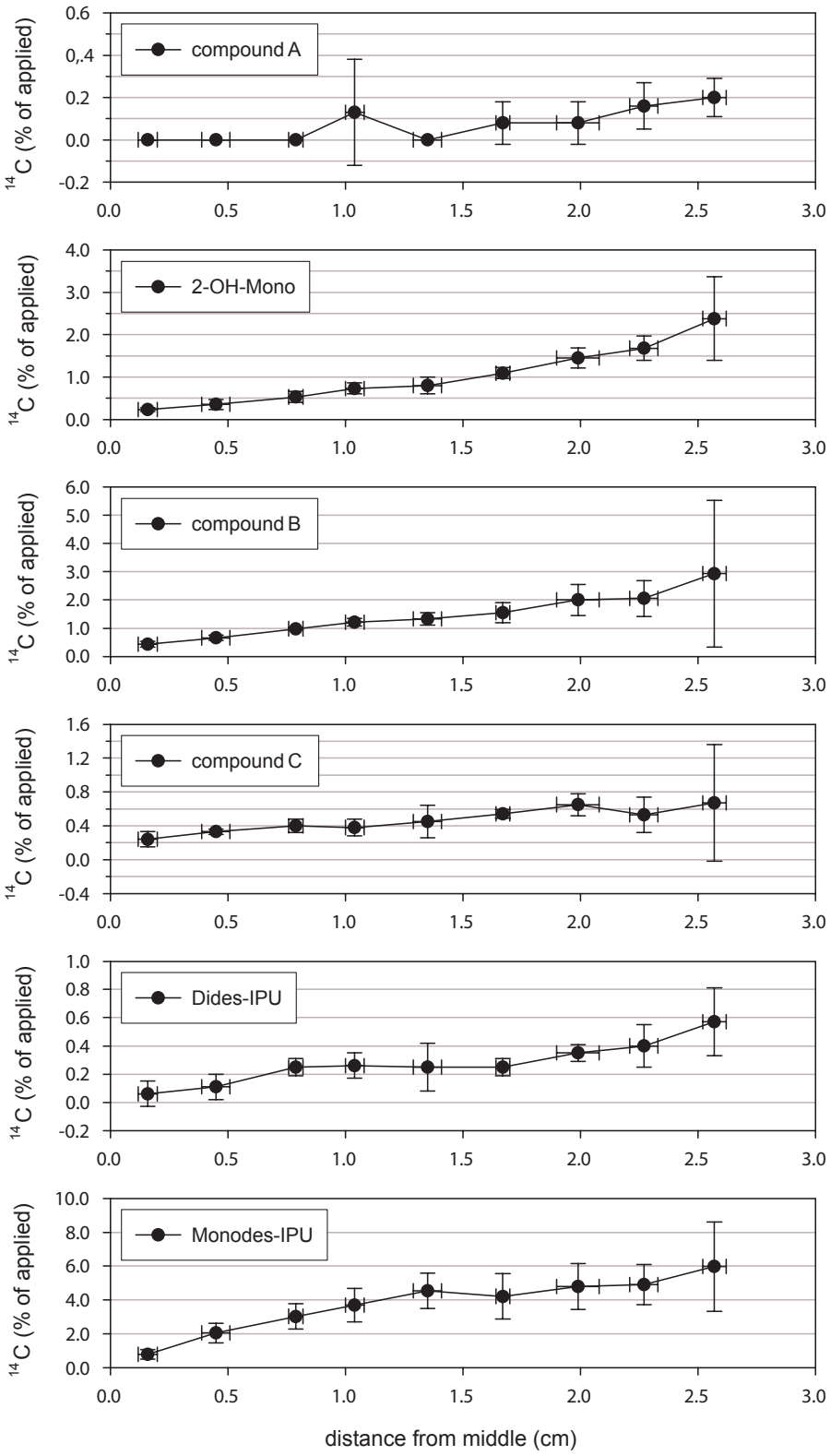


Figure 3.22a: ASE extractable compounds identified and quantified by HPLC. The values are depicted versus distance from the microbial hot-spots in the middle of the diffusion tubes. Values are means of four samples pooled from the two sides of the concentration profiles of two experimental replicates. Error bars indicate standard deviation. Compounds A, B, C could not be identified because standards were not available.

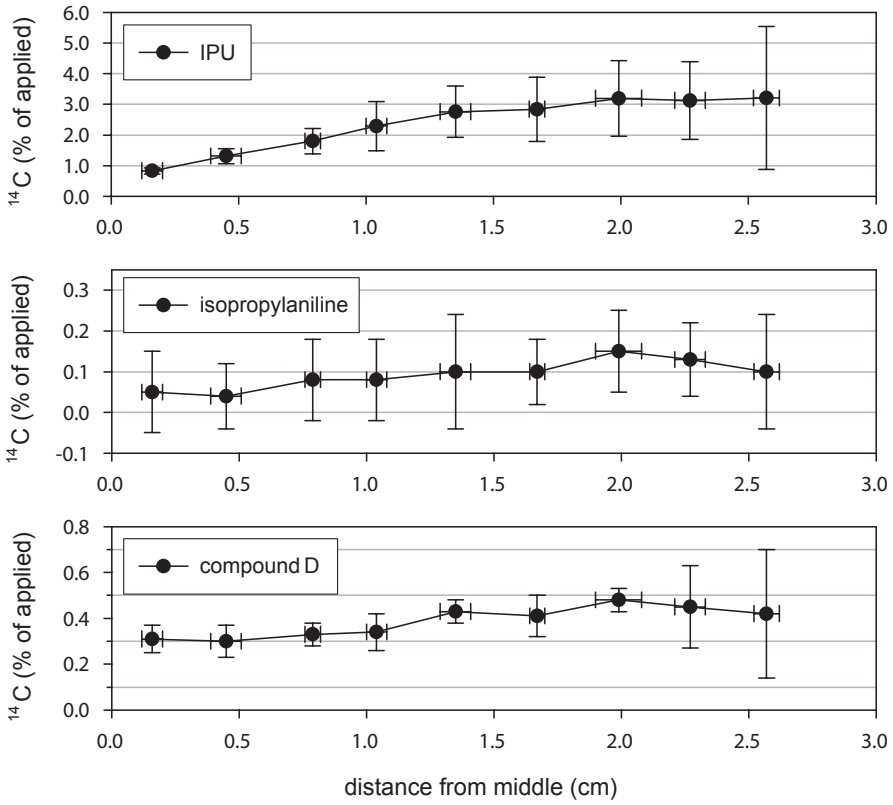


Figure 3.22b: ASE extractable compounds identified and quantified with HPLC. The values are depicted versus distance from the microbial hot-spots in the middle of the diffusion tubes. Values are means of four samples pooled from the two sides of the concentration profiles of two experimental replicates. Error bars indicate standard deviation. Compound D could not be identified because a standard was not available.

hydroxy-isoproturon, peak B), and 3-[4-(1-hydroxypropan-2-yl)phenyl]-1,1-dimethylurea (1-hydroxy-isoproturon, peak C).

Table 3.5: Correlation of concentration of ASE extractable compounds (compare figure 3.22) with distance from microbial hot-spots. Nonparametric correlation coefficients (Spearman-Rho) are given.

A	2-OH-Mono	B	C	Dides-IPU	Monodes-IPU	IPU	isopropyl-aniline	D
0.575*	0.936*	0.835*	0.359*	0.644*	0.743*	0.617*	0.245	0.312*

* significantly ($\alpha = 0.05$) different from 0

The most abundant compound was Monodes-IPU followed by the parent compound isoproturon, 2-OH-Mono, and compound B (figure 3.22). Concentrations were significantly ($\alpha = 0.05$) and positively correlated with distance from microbial hot-spots with the exception of isopropylaniline (table 3.5). Compounds measured in low quantities showed only a relatively weak correlation whereas more abundant compounds were more strongly correlated with dis-

tance. However, distinct concentration profiles were observed (even isopropylaniline concentrations showed this tendency) which is consistent with diffusion towards the microbial hotspots.

Chapter 4

Discussion

4.1 Diffusion of DOM and inorganic nitrogen in free soil solution

The experimental setup proofed to be suitable for studying diffusion in free soil solutions. It was possible to study diffusion of single substances and DOM fractions in this very complex matrix. Soil solutions with typical DOC contents of about 20 mg (carbon) L^{-1} can apparently be seen as dilute solutions since diffusion coefficients of two species matched theoretical values for infinite dilution. Consequently it can be assumed that electrostatic interactions between diffusing molecules and ions were limited. However, for several substances some kind of interaction with other molecules or ions was apparent from the results. Therefore application of more complex diffusion theories such as the Maxwell-Stefan diffusion model (Kerkhof and Geboers, 2005) would be appropriate. However, these theories require knowledge about all diffusing substances, even those at low concentrations. It is simply impossible to know all solutes of a soil solution. Thus the classical diffusion laws (Fick, 1855) had to be used. The diffusion coefficients reported here should not be used for predicting diffusion fluxes, but should be seen as more empirical parameters that allow to compare diffusion characteristics of compounds in the solutions.

4.1.1 Ammonium and nitrate diffusion properties in plant and soil extracts

Inorganic nitrogen is a crucial nutrient for plants as well as soil microbes. It easily can become a limiting factor of biological processes in soil. Therefore application of inorganic nitrogen fertilizers is an indispensable agricultural practice in order to achieve high crop yields. Diffusion processes are heavily involved in the nutrient supply of plants and microbes.

Experimental results in this work showed a much lower diffusion coefficient of ammonium than the theoretical value. This indicates that diffusion of ammonium in soil solution is influenced by other substances in the solution. A possible mechanism would be a diffusion drag due to electrostatic interactions with negatively charged substances, e. g., humic substances. But if such effects were significant in the rather dilute solutions which were studied, one would expect the diffusion coefficients of the anions hydrogen carbonate and nitrate also to be effected by this mechanism. Instead their measured diffusion coefficient matched the theoretical values excellently. Cussler (1997) points out that association of solutes influences their diffusion characteristics. Monovalent cations such as NH_4^+ are known to associate with humic and fulvic acids and form soluble salts thereby increasing the solubility of humic substances (Norman et al., 1987). It is therefore assumed that part of the ammonium ions associates with organic substances while the other part stays in the dissociated form. This could, depending on the dissociation constant, substantially slow down the diffusional flux of ammonium. Experimental observations are in good agreement with this concept. As a consequence diffusion of ammonium can only be accurately described by a model that takes into account association. To apply such a model the dissociation constant would have to be known or sufficient data would have to be available to fit this parameter. Since neither is the case in this study, the diffusion coefficient of ammonium given in the results is not accurate and more of an empirical parameter. Nevertheless it is adequate for comparison with other substances of the soil solution.

Diffusion properties of nitrate and ammonium were independent of the origin of the solution. For nitrate this could be expected; for ammonium this is somewhat surprising if diffusion of ammonium is influenced by diffusion of humic-like substances. However, the ratio of ammonium to DOC was relatively constant for all original solutions. Thus the identical results in all solutions do not contradict the theory.

From the results it can be concluded that nitrate diffuses about twice as fast as ammonium in the presence of humic substances. Therefore NO_3^--N is transported much faster than NH_4^+-N on the microscale in soils. Humic substances thus decrease the bioaccessibility of NH_4^+-N, which amplifies the difference to NO_3^--N already caused by the stronger fixation of ammonium to clay minerals (Scheffer et al., 2002). On the other hand ammonium diffusion is still faster then diffusion of DOM molecules which means free inorganic nitrogen is more mobile than free soil organic carbon.

4.1.2 Dissolved organic carbon and dissolved organic nitrogen diffusion properties in plant and soil extracts

It is rather difficult to study diffusion of DOM as it is a complex mixture of substances which are mostly unidentified. It is not possible to determine one diffusion coefficient, as each of the mostly unknown substances has its own distinct diffusion properties and the substances most likely interact with each other and other (mostly ionic) substances during diffusion. Thus the diffusion coefficients for DOC and DON determined in this study should be seen as empirical parameters which allow to compare diffusion velocities of different substance classes, but should not be used to predict diffusion fluxes.

The most important parameter affecting diffusion properties of a molecule is its size. Large organic matter molecules such as humic substances can be expected to move more slowly by diffusion than simple organic substances such as sugars or amino acids. Humic substances are known to build aggregates in solution (Caceci and Billon, 1990) and as a result diffusion of humic matter can be expected to be slow. However, filtration before the experiments would have removed most of these aggregates.

In this study only minimal influence of origin on diffusion of DOC was observed. This is surprising because one would expect that the different composition of plant extracts, which unlike soil extracts should not contain humic substances, would lead to unequal diffusion characteristics. Kelleher and Simpson (2006) using NMR approaches found no evidence that the operationally defined soil humic acids belong to a distinct chemical category. They conclude that humic substances are a complex mixture of plant and microbial biopolymers and their degradation products. It follows from their work that plant extracts might contain molecules very similar to soil humic substance molecules.

The calculated diffusion coefficients of DOC in soil and barley plant extracts were all approximately equal to $0.27 \cdot 10^{-5}\,cm^2\,s^{-1}$ (4 °C) which is in the same range as the diffusion coefficient of sucrose (Cussler, 1997). Pinheiro et al. (1996) used dynamic light scattering and voltammetry to measure diffusion coefficients of humic acids (0.45 µm filtrated). These diffusion coefficients ranged from 1 to $5 \cdot 10^{-8}\,cm^2\,s^{-1}$ (4 °C, converted from 25 °C values using equation 2.4). However, their sample preparation involved the standard humic acid extraction procedure with KOH and HCl. Thus their samples did not reflect the naturally dissolved humic substances and were possibly strongly altered in their chemical structure. Still, if one assumes that humic substances exhibit such low diffusion coefficients, it follows that the natural DOM,

which was studied here, contained only minor amounts of humic acids. This agrees with DOM being the most labile organic matter pool in soil. The average size of the organic molecules calculated from the DOC diffusion coefficient with the Stokes-Einstein equation (see section 2.6.1, equation 2.3) is 1.8 nm and therefore much smaller than the mean size of humic acids which are in the dimension of 100 nm (Pinheiro et al., 1996). On the other hand by means of fluorescence correlation spectroscopy Lead et al. (2000) found diffusion coefficients of about $0.14 \cdot 10^{-5}$ cm^2 s^{-1} (4 °C, converted from 25 °C values using equation 2.4) for humic and fulvic acid standards obtained from the International Humic Substances Society (Suwannee River). This is relatively close to DOC diffusion coefficients observed here and would allow the possibility of substantial amounts of humic substances in the soil solutions.

The diffusion coefficient of DON in soil "Cambisol 1" matched the diffusion coefficient of DOC excellently. This indicates that the quality of nitrogen containing substances was very similar to the quality of total organic substances, i. e. mainly substances more stable in soil contributed to the DOM pool. This can be expected in an agriculturally used soil, which had not been fertilized for several months and is in agreement with the high DOC : DON ratio of 14.5. Although results are not statistically reliable for DON in soil "Cambisol 2" DON diffusion seems to be faster than DOC diffusion. This indicates that DON comprises more smaller and therefore more labile organic substances in this soil. Supporting this is the DOC : DON ratio of the extract which was with a value of 5.2 much lower then in "Cambisol 1" extract. This can be expected in a soil shortly after fertilization. In barley plant extract DON was transported about 50 % faster by diffusion than DOC. Again this shows that mainly smaller and more labile compounds such as amino acids were part of the DON pool in this extract. The DOC : DON ratio in the plant extract of 8.3 was slightly higher than in "Cambisol 2" soil extract, where nitrogen content was strongly elevated by fertilization.

DON diffusion characteristics seem to depend much more on the degree of humification of soil DOM than DOC diffusion characteristics. The mean diffusion coefficient of DON appears to be strongly influenced by DON quality (DON in humic substances versus DON in more labile organic substances) as deduced from soil history and origin of extract. This quality seems to be somewhat correlated with DOC : DON ratio.

Although plant extract may contain biopolymers with similar diffusion characteristics as humic substances, most of their nitrogen seems to be part of smaller molecules. This means that DON from fresh plant litter is more mobile than litter DOC. This further enhances de-

pletion of nitrogen in plant litter, in addition to the better solubility of nitrogen containing plant compounds which was observed in barley plant extracts compared to the original plant material.

4.1.3 Fluorophores in soil and plant extracts and their diffusion characteristics

Fluorophore groups which are regarded as indicators of humic substances (Stedmon and Markager, 2005; Ohno and Bro, 2006) were present in all extracts. It should be stressed that these fluorophores are not per se humic substances, but are typical present in humic or fulvic acid samples. Therefore they are called humic-like or fulvic-like. Substances that might contribute to these fluorophore groups are coumarines, quinones, 2-aminobenzoic acid, salicylic acid and several others (Ma and Green, 2008). Since these fluorophore groups were also measured in the plant extract they are clearly not only indicators of humic substances, which are not present in plants, but have to be seen as indicators of molecules of high complexity which are precursors of humic substances, i. e. biopolymers.

Relative abundance (per mg DOC) of humic-like and fulvic-like fluorophores was highest in soil "Cambisol 1". Again this is consistent with the long time period without fertilization. In contrast DOM from soil "Cambisol 2" which had been amended with organic fertilizers a short time before sampling showed a much smaller portion of humic-like and fulvic-like fluorophores. In fact, even barley plant extract exhibited more fluorescence in the associated wavelength regions. Although fluorescence data clearly show that substances that are seen as indicators of humification in soil were present, the determined diffusion coefficients of DOC and DON indicate that large molecules like humic acids or biopolymers might account only for a minor fraction of DOM in the (filtrated) extracts (see section 4.1.2).

Tryptophan-like fluorophores were present to a greater extent only in the plant extract. Nevertheless, although signals were relatively weak, these fluorophores could be clearly detected in the soil extracts where they were present at equal relative concentrations. Because their abundance was not different between the soil extracts, although one soil had been fertilized recently and the other soil not, and the soils were both bare during sampling, it can be assumed that tryptophan in these soils is mainly of microbial origin and not from plants or organic fertilizers. Organic fertilizers contain substantial amounts of tryptophan, but the majority can be fixed in proteins (Arkhipchenko et al., 2006).

Results showed that there were no significant differences between diffusion properties of fluorophores between soils. Diffusion of the tryptophan-like fluorophores in soil solution was significantly and considerably slower than the theoretical value for tryptophan at 4 °C of $0.39 \cdot 10^{-5}$ cm^2 s^{-1} (see section 3.1.2). There are two possible reasons for this observation: Either other molecules with higher molecular volumes were part of this fluorophore group or some of the fluorescing molecules were associated with other molecules and built aggregates. Since association would cause a wavelength shift of the fluorescence maximum or could even suppress fluorescence the former seems more likely.

In barley plant extract diffusion of tryptophan-like fluorophores was much faster than in soil extracts. The diffusion coefficient was even higher than the theoretical value of tryptophan, but standard deviation was relatively high and the difference not significant. In fact two out of three measured values (with acceptable balances) were identical and with $0.37 \cdot 10^{-5}$ cm^2 s^{-1} a very good match of the theoretical value for tryptophan. It is therefore likely that this fluorophore in the plant extract actually is tryptophan. Thus, while this fluorophore in soil solution is only an indicator of protein-derived substances, it might be a specific measure of the amino acid tryptophan in barley plant extracts. Interestingly mean diffusion coefficients of DON in "Cambisol 1" extract and barley plant extract were very close to values for tryptophan-like fluorophores. Amino acids are the largest pool of water soluble organic nitrogen in soils (Stevenson, 1982) and tryptophan is the biggest and therefore most slowly diffusing proteinogenic amino acid.

Diffusion characteristics of humic-like and fulvic-like fluorophores were very similar in all extracts. A slight increase of diffusion coefficient was observed in the order "Cambisol 1" to "Cambisol 2" to barley plant extract, but this increase was not significant. This means that composition of these fluorophore groups was likely similar in soil extracts and plant extract. This is somehow surprising because these fluorophores are widely used to assess the degree of humification of DOM. The results show that not only exhibit plant extracts fluorescence peaks in this region typical for humic substances but these substances have also very similar diffusion characteristics. It is therefore possible that these substances are introduced to soils with plant litter and then are recalcitrant or stabilized and can remain unchanged for a relatively long time (von Lützow et al., 2006).

Humic-like and fulvic-like fluorophores showed a trend to be (fluorophore group 1) or were even significantly (fluorophore group 2) slower than the mean diffusion flux of DOM. Thus, these substances were indeed more complex than the average DOM.

Overall, diffusion characteristics of all fluorophore groups were remarkably similar with the exception of tryptophan-like fluorophores in the plant extract.

4.2 Diffusion in the detritusphere - the source system

In principle the experimental system proved to be suitable for studying diffusion processes in conjunction with degradation of organic litter materials in soil. However, due to the problems in ensuring a stable water content, the setup should be improved for further experiments. Most importantly aeration using a vacuum pump should be avoided. A possible alternative would be to rely on diffusion of ^{14}C-CO_2 towards a strong CO_2-absorber. Such a system would work best if the gas space of the incubation vessels is small. Therefore it is advisable to use a separate incubation vessel for each replicate. This would also provide statistical data about mineralization. On the other hand separate mineralization measurements are more costly in lab space, work and monetary investments.

The high radioactive label of the litter allowed to detect very low concentrations of litter-derived carbon in soil. Elevated ^{14}C-concentrations were measured even relatively far from the source. Thus it was possible to survey diffusion profiles with a good accuracy. A high number of measurements per profile was available which ensures statistically reliable results.

Dried and ground plant litter was an excellently suited material for the studies. It could be distributed evenly in soil aliquots which lead to excellent ^{14}C balances. ^{14}C-labeled microbial biomass was more problematic in handling than plant litter. Because of the low amounts it was not possible to grind it using a ball mill. As a result distribution was not as uniform between replicates and ^{14}C balances could not be established. Still this did not overly affect quality of the experimental results. Differences to plant litter can be explained with the weaker ^{14}C label of the microbial biomass. It might be possible to achieve a more even distribution of the microbial biomass if it is not dried before application. However, this would impair handling during application.

4.2.1 Bioavailability of plant and microbial biomass

As microbial biomass and plant biomass differ in composition they differ in total bioavailability as well as in proportions of labile and recalcitrant compounds (Kögel-Knabner, 2002): Cell walls of fungi consist of structural polysaccharides, particularly chitin and β-glucan, which are non-soluble in water. Fungal cell matrix consists mainly of mostly water soluble polysaccharides. Fungi and also some bacteria synthesize melanins which are either incorporated in the cell walls or occur as an outermost layer. These melanins protect the fungal cell wall against hydrolytic enzymes (Butler and Day, 1998) and thus hinder decomposition. It is sometimes assumed that melanins are precursors of humic substances (Saiz-Jimenez, 1996). A major part of bacterial cell walls is murein, a peptidoglucan which contains carbohydrate as well as amino acid elements (Koch, 1990). Murein amounts to about 50 % of dry mass of cell walls of Gram-positive bacteria but only 10 % of dry mass of cell walls of Gram-negative bacteria. The bacterial cell wall polysaccharides are relatively easily decomposed, but their basic units accumulate during litter decomposition (Kögel-Knabner, 2002).

In a less detailed approach microbial biomass is regarded as a composition of three microbial products: extracellular polymeric substances, soluble microbial products, and recalcitrant biomass (Laspidou and Rittmann, 2002). Functions of the extracellular polymeric substance include adhesion to surfaces, aggregation of bacterial cells in biofilms and formation of a protective barrier. Water solubility of these substances is very limited. Soluble microbial products are produced during substrate utilization or set free from lysis of dead cells. They are the most bioavailable part of biomass. The most recalcitrant fraction is build by insoluble residues of dead cells. Due to the treatment of the ^{14}C-labeled biomass in this study, particularly autoclaving, before the experiment (section 2.3) it is assumed that the biomass contained only minor amounts of easily soluble substances. Due to the extracellular polymers and denaturation from heat treatment the biomass build a very hard composite and probably only the outside of the applied particles was accessible to microbes in the beginning of the experiment. Furthermore only part of the soil microbial population might be able to degrade the polymers. Consequently mineralization results indicate a lower bioavailability of the ^{14}C microbial biomass than of barley plant material.

Intracellular and storage materials of plant biomass generally exhibit a good solubility in water and are highly bioavailable and therefore labile in soil. These substances include proteins, starch, fructans and chlorophyll. All of these compounds can be degraded by a high

number of microbial species (Kögel-Knabner, 2002). Barley plant litter contains a significant proportion of water soluble organic substances. 11.8 % of carbon in unlabelled barley plants in this study and 27.3 % of the nitrogen were soluble (compare sections 2.9.1 and 3.1.1). Cellulose which is not water soluble and decomposed slowly under aerobic conditions is the most abundant biopolymer in plants. Most cellulose-decomposers are fungi, but also many bacteria can degrade cellulose. The other polysaccharides of the plant cell wall, hemicelluloses and pectin, can also be degraded by many bacteria and fungi. Their decomposition rate is higher than that of cellulose (Swift et al., 1979). The very recalcitrant lignin can be mineralized only by white-rot fungi. Other fungi may induce structural changes but not complete mineralization. Therefore lignin is mostly degraded by consortia of microorganisms (Haider, 1992). Lignin is a large contributer to soil organic matter residues. Sun et al. (2002) found 37.5 % (mass : mass) cellulose, 36.1 % hemicelluloses, 15.5 % lignin and 2.5 % waxes in barley straw. Other plant biomass compounds are tannins, though of minor importance in grasses, lipids and the more easily degradable cutin and suberin (Kögel-Knabner, 2002).

4.2.2 Mineralization of litter derived carbon

There are two main factors which may limit mineralization of litter in soil: bioavailability of the substrate and the size of the microbial population. The aforementioned characteristics of the two substrates used in the experiment cause distinct differences in short-term and long-term bioavailability. Because of very different soil characteristics, particularly different water retention curves, the soils' microbial community size and composition was most likely appreciably different.

Mineralization results for barley in soil "Cambisol 1" show a decline of the rates after considerably less than 2 days. Presumably all easily soluble compounds had been decomposed. This indicates that mainly less soluble substances were degraded in the later stage of the experiment and mineralization was limited by bioavailability. A similar observation was made for barley litter mineralization in soil "Arenosol". In this soil the maximum rates were not reached until about 2.5 days. It follows that in this soil microbial population size was limiting initially and only after most easily soluble substances were decomposed bioavailability of the litter carbon became limiting.

It is widely accepted that organic matter has to pass through the dissolved organic matter pool in order to be mineralized by microorganisms (Schimel and Weintraub, 2003). While

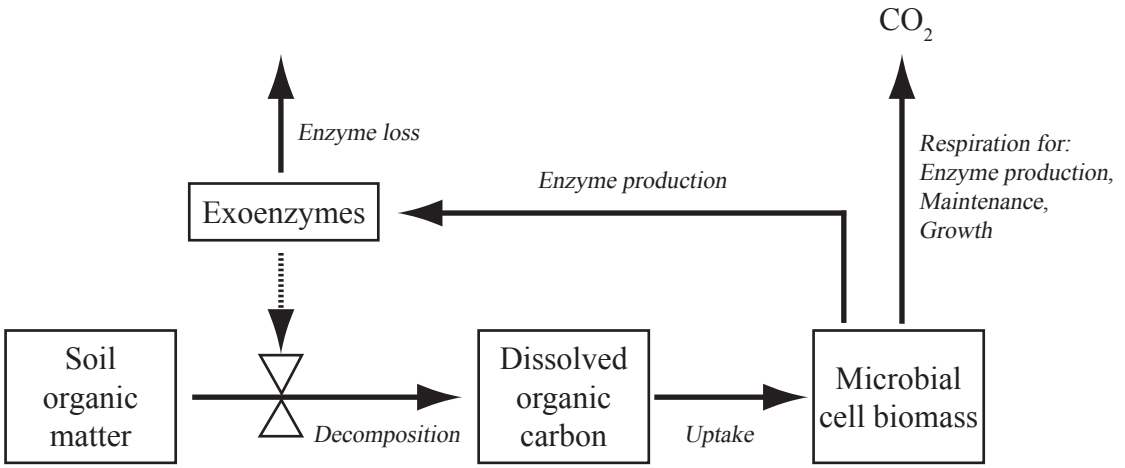


Figure 4.1: Model of C turnover in soil (Schimel and Weintraub, 2003). Solid lines indicate flows of material, dashed line connected to the "valve" indicates regulation point. Organic carbon has to pass through the DOC pool to be utilized as microbial substrate. Decomposition of insoluble carbon is regulated by exoenzymes.

abiotic processes like oxidation or hydrolysis can play a role, action of extracellular enzymes is the dominant process for conversion of UD-SOM to DOM (Kuzyakov et al., 2008). Schimel and Weintraub (2003) suggested a simple model for the regulation of SOM decomposition by exoenzymes (figure 4.1). This regulation gets most important during a second phase of plant litter decomposition when soluble litter-derived organic carbon has been mineralized. High microbial growth rates due to the easily available substrate during the first phase of decomposition lead to a high potential of enzyme production. Still the transformation of UD-SOM into DOM is limiting mineralization rates in the second phase. As the amount of plant litter residues in soil decreases, the bioavailability of the organic matter decreases. As a result microbial numbers slowly decrease while more and more exoenzymes have to be synthesized by microbes in order to sustain substrate supply. This theory agrees well with the slowly declining mineralization rates which were observed after the initial peak.

The effect of grinding on litter decomposition is somewhat unclear. The general observation is that a decrease in particle size results in increasing decomposition rates (Dickinson and Pugh, 1974). This is believed to be due to better access of exoenzymes to the litter material (Swift et al., 1979). Sørensen et al. (1996) found a strong positive effect of grinding on microbial biomass development in litter degradation experiments. By contrast, other studies observed no effect of grinding on microbial biomass (Vestergaard et al., 2001; Ambus and Jensen, 1997). It

could be the case that ground and unground plant residues exude easily soluble and more labile substances at similar rates. This would mean there are only small differences between ground and unground material in decomposition during the initial stage. Only later, when mainly less soluble substances are fed on, differences in access of enzymes to these substances, i. e. surface area of the litter particles, become important.

There are two possible explanations for the observed development of mineralization rates of ^{14}C microbial biomass. Microorganisms which were able to degrade the ^{14}C biomass might have been not abundant in the beginning of the experiment. Thus mineralization would have been limited by growth of these microorganisms. Mineralization rates would start declining as soon as the easily degradable substances have been utilized and bioavailability of the microbial substrate decreases. The other explanation is that accessibility limited mineralization of ^{14}C in the beginning. The agglutination of the ^{14}C biomass would result initially in small surfaces. Degradation of the polymers would then lead to increasing surfaces, which would accelerate the degradation process. Again rates would decline when the easily degradable substances have been utilized.

The results clearly show that the applied microbial biomass was initially less bioavailable than plant litter. Possible reasons are an inherent lower availability of microbial biomass due to lower water solubility and higher molecular complexity, the bigger particle size or effects of heating for sterilization. The microbial biomass was harvested from liquid culture when ^{14}C mineralization rates declined in this culture. Therefore it is not expected to contain highly available substances as does plant litter. While in the early stage fundamental differences were apparent, in the later stage of mineralization bioavailability of plant litter and microbial residues does seem to decline in similar ways.

Cumulative mineralization of ^{14}C biomass in soil "Arenosol" was much lower in the end then in all other treatments. This is most likely an artifact from drying. In this treatment the soil dried to a much higher value of water tension whereas all other treatments dried to comparable water tensions.

Mineralization of litter in soil profits from diffusion processes the most in the beginning of substrate degradation when there are significant amounts of soluble substances. Large amounts of soluble substances cannot be degraded immediately at the source and are transported further into the soil where they are mineralized. Gaillard et al. (2003) found that one fourth to one third of short-term mineralization of plant litter carbon took place in the soil ad-

jacent to the litter. As microbial growth takes place at the substrate source diffusion of original litter-derived substances diminishes. Instead microbial degradation products are transported away from the hot-spots of highest microbial activity at the litter. This leads to a succession of microbes where the microbes distant from soil have to feed on less bioavailable substrates of less nutritional value (Poll et al., 2006). Consequently the highest microbial growth takes place in close vicinity of the litter.

4.2.3 Effect of litter introduction on soil microbes

As can be expected results showed an increase of microbial biomass close to the applied litter after four weeks. After such a long time under favorable conditions for degradation it can be assumed that only small amounts of original litter material, e. g. lignins from the plant material, were still existent. Cumulative mineralization of close to 50 % of original litter carbon supports this assumption. It is unlikely though not impossible that results from chloroform fumigation-extraction (see section 2.8) were affected by enhanced extraction of original litter carbon after fumigation. However, increased microbial biomass was observed up to a distance of about 1 cm from the litter. This indicates that indeed nutrients were transported out of the litter into the adjacent soil.

Although steep gradients of total ^{14}C concentration were observed the proportion of microbial biomass ^{14}C can be seen as constant. This could be an indication that the system was in a kind of equilibrium at sampling time. Mineralization results show that easily available substances were not present anymore. The amount of living microbial biomass is mainly governed by substrate amount and availability. It is likely that at this late stage of degradation availability of ^{14}C labeled substrate was the same regardless of distance from point of application. A possible exception could be the point of application as there the most recalcitrant residues of litter might still be present. But even there mineralization of soil microbial carbon most likely predominated. Since easily degradable organic litter substances were not present anymore diffusion of DOM had decreased to very low or even nonexistent fluxes. The concentration profiles probably developed at earlier stages when more water soluble ^{14}C was present. They were somewhat conserved by the decreasing water solubility of litter-derived carbon which remained in soil.

Poll et al. (2006) found that fungi assimilated carbon directly in the litter, while bacteria relied more on transport. This is consistent with other studies which show that less bioavail-

able litter compounds like lignocellulose favor fungi compared to easily degradable substances which are mineralized by fast growing bacteria (Frankland, 1992; Snajdr et al., 2008). Poll et al. (2006) further state that the spatial dimension of the detritusphere - the distance up to which size or composition of the microbial community are influenced by litter added to a soil - is determined by the ratio of decay rate to transport rate. Therefore even if soils show very different diffusion characteristics the detritusphere can be of similar size if mineralization rates differ in the same way. In the study presented here results indicate for both substrates that this seems to be the case for the soils studied. This can be expected because as a general rule easily soluble small natural organic molecules which are transported faster by diffusion tend to be more bioavailable due to their solubility and chemical structure.

Ettema and Wardle (2002) point out that spatial heterogeneity of microorganisms on the microscale is often correlated with substrate heterogeneities. The experiments conducted in the study presented here simulate development of microbial heterogeneity from substrate heterogeneity. Clear differences in microbial biomass and therefore microbial numbers were observed depending on distance from litter which had been introduced into soil. As discussed above composition of microbial community can also be expected to show heterogeneity depending on litter quality and stage of decomposition. The results showed that due to diffusion microbial hot-spots do not have a clear-cut boundary but microbial numbers and community composition change gradually.

4.3 Diffusion and degradation of pesticides in soil - the sink system

The experiments on source induced diffusion gave insight in the mechanisms that lead to heterogeneities of the soil microbial population and the resulting soil functions. These heterogeneities can cause sink induced diffusion which was studied on the example of degradation of the herbicide isoproturon by soil microbial hot-spots. Similar experimental problems occurred as in the experiments on source induced diffusion. However, overall the experiment was successful. Results showed that transport towards the microbial hot-spots took place and ^{14}C was accumulated there, probably as degradation products and microbial cell detritus.

4.3.1 Mineralization of isoproturon

Results show enhanced isoproturon degradation in the soil columns with isoproturon degraders applied as a layer in the middle compared to the soil without these degraders. The

maximum rates (about 1 % d^{-1}) in the former were two times the relatively constant rates (about 0.5 % d^{-1}, Kühn, 2004) observed in the latter. This means that the microbial degraders could enhance degradation of the pesticide at least initially (during the first 3 weeks). Constant mineralization rates indicate cometabolic degradation, i. e. the pesticide is degraded due to its susceptibility to microbial enzymes produced for degradation of natural substrates. Rates of cometabolic degradation are mainly determined by the natural substrate and initial size of the microbial population (Johnson et al., 2004). Since the degradation of the cosubstrate does not contribute to microbial growth, degradation rates of this cosubstrate, such as isoproturon, are often constant in soil. In contrast degradation kinetics (observed as mineralization) in the degradation-diffusion experimement clearly indicate microbial growth and consequently at least part of the isoprotron degradation was due to metabolic degradation, i. e. isoproturon was utilized as a substrate by the degraders added to the soil.

In a second phase of the "sink diffusion" experiment mineralization rates were almost identical to those in the soil without degraders added. It can be concluded that at this point no significant amounts of ^{14}C labeled substrate were transported to the expanded clay particles with active degraders anymore. After two measurements where mineralization rates matched the native cometabolic isoproturon degradation capacity of the soil almost exactly mineralization rates dropped down significantly. It is suspected that drying occurred as a singular event during aeration at this time (30 days). The decrease of mineralization can be predicted using the equation presented by Schroll et al. (2006):

$$\frac{CM}{CM_{pF2.18}} = \frac{\theta - \theta_{pF5}}{\theta_{pF2.18} - \theta_{pF5}} \tag{4.1}$$

where CM is cumulative mineralization and θ is volumetric water content. In case of constant rates as observed for cometabolic degradation the same relationship holds true for the mineralization rates. For cometabolic degradation of isoproturon in soil "Cambisol 3" the observed drying during the "sink diffusion" experiment would theoretically result in a reduction of the mineralization to 30 % of the optimum value (0.15 % d^{-1}). The mineralization rates in the end of the experiment (about 0.25 % d^{-1}) are 50 % of the mineralization at pF 2.18 and thus slightly higher then the predicted rate but similar.

Grundmann et al. (2007) reported that an even distribution of hot-spots of the degraders (established on expanded clay particles) in the same soil resulted in 53 % cumulative miner-

alization after 46 days. They present strong evidence that mineralization was not limited by diffusion if about 20 clay particles were mixed with 50 g (dry mass) of soil "Cambisol 3". Cumulative mineralization during the "sink diffusion" experiment after 43 days was with 25 % significantly and considerably lower. This proves that mineralization was diffusion limited as intended with the experimental setup.

4.3.2 Bioavailability of isoproturon in soil

Probably the most important parameter of bioavailability of pesticides in soil is their sorption behavior. Sorption of pesticides in soil may be divided into two processes. One is short-term sorption which is investigated by batch experiments. However, although sorption isotherms obtained from these experiments are often assumed to describe instantaneous sorption in fact they only show the equilibrium state. Equilibrium is reached when adsorption and desorption rates are equal. Since desorption in soil is slower than adsorption (Beulke et al., 2004) desorption kinetics determine equilibrium and it can take considerable time until equilibrium is reached. In this study equilibrium in batch experiments was reached after about 3 days. The sorption isotherm (section 2.7) indicates that appreciable amounts of isoproturon stay in solution, ensuring a high bioavailability of the herbicide during the initial phase of degradation experiments. Later the desorption rate constant is a limiting factor of degradation (Beulke et al., 2004).

Additionally to short-term sorption slower processes remove the pesticide from the most bioavailable, dissolved pool. These processes include diffusion into soil aggregates, uptake into soil microbes, and (partial) degradation and take generally weeks to months until they reach equilibrium (Cox and Walker, 1999). Degradation products of isoproturon may be less bioavailable or even resistant to microbial degradation. Moreover these degradation products can be incorporated into SOM molecules and thereby become bound residues. The bioavailability of bound residues is extremely low and therefore they pose only minimal immediate environmental risks. However, because of their low degradation rates bound residues can accumulate and eventually be set free when soil conditions change (Barraclough et al., 2005). Therefore bound residues are not a desired end product of degradation and full mineralization should be achieved after application of pesticides to soil.

Isoproturon degradation in soils is known to result in considerable amounts of bound residues (Reuter et al., 1999). Some degradation products such as isopropylaniline can bind

to precursors of humic substances (Reuter et al., 1999) and form non-degradable compounds such as 4,5-bis-[4-(propan-2-yl)phenyl]aminocyclohexa-3,5-diene-1,2-dione (Sørensen et al., 2003). A high proportion of bound residues is to be expected if degradation takes place in several steps, i. e. the parent compound is not mineralized directly. Thus the highest proportions of bound residues are observed in soils where the pesticide is degraded via cometabolism.

Bound residues are commonly measured as non-extractable residues in degradation experiments with labeled substances. Originally they were defined by the International Union of Pure and Applied Chemistry (IUPAC) as "chemical species originating from pesticides, ..., that are unextracted by methods which do not significantly change the chemical nature of the residues." Later a modified definition has been widely accepted: Bound residues are "compounds in soils, plants or animals which persist in the matrix in the form of the parent substance or its metabolite(s) after extraction. The extraction must not substantially change the compounds or the structure of the matrix" (Gevao et al., 2000). As a result measured proportions of bound residues depend on the extraction method; in case of isoproturon this is methanol extraction. A serious problem is that non-extractable residues include labeled remains of microbial biomass which are not problematic from the environmental perspective. This measure therefore only gives the apparent bound residues. To distinguish bound residues from the apparent bound residues sophisticated methods such as nuclear magnetic resonance (NMR) techniques or harsh extractions followed by chromatographic or spectroscopic methods have to be applied (Northcott and Jones, 2000).

In the "sink diffusion" experiment only the apparent bound residues were determined, because bound residues are not transported by diffusion and apparent bound residues may be regarded as one pool of ^{14}C which is fixed in soil and not available for transport. In order to accurately model the system bound residues would have to be analyzed but this is beyond the scope of this thesis. In the end of the experiment most of the applied ^{14}C was found as apparent bound residues. Since significant amounts of ^{14}C were measured in living microbial biomass it is assumed that a good part of the unextractable residues was microbial remains. Still it is likely that a significant proportion was bound residues as the parent compound isoproturon and its metabolize could still be extracted. Furthermore cumulative mineralization was only 25 % and thus it is unlikely that much more than the same amount of ^{14}C would be part of living or dead microbial biomass. In numerous studies it has been shown that 25 to

75 % of ^{14}C applied as isoproturon are converted to bound residues during 2 to 3 months of incubation in soil.

In conclusion the large amount of unextractable residues supports the assumption that outside of the added expanded clay particles isoproturon was only degraded cometabolically. Since only a small proportion of ^{14}C was part of free isoproturon and free metabolites this cometabolic degradation might be strongly connected to humic substance turnover.

4.3.3 Metabolic pathways of isoproturon degradation

Degraders of isoproturon utilize the pesticide primary as a carbon source. Several metabolites can result from partial degradation of isoproturon in soil (figure 4.2). The most important and likely rate limiting degradation step is the breakdown of the dimethylurea side chain. The resulting metabolite isopropylaniline (4-(propan-2-yl)aniline) is generally degraded fast in soils by oxidative deamination and different ring cleavage pathways (Parris, 1980). However several substitutions to the phenylring, reaction with the potential humic precursor catechol to a trimer (step 14 in fig. 4.2), or polymerization (step 15 in fig. 4.2) may delay or prevent its mineralization (Sørensen et al., 2003).

Other degradation pathways include hydroxylation of the propyl side chain. 3-[4-(1-hydroxypropan-2-yl)phenyl]-1,1-dimethylurea has been reported as a dead-end product, but its environmental significance is unclear (Sørensen et al., 2003). However the other hydroxylated metabolites have been frequently detected in different soils (Sørensen et al., 2003) but information about this pathway is still limited. It is unknown if and how 2-(4-aminophenyl)propan-2-ol is mineralized in soil.

In the soil where the microbial community used in the "sink diffusion" experiment originates from only the parent compound and the single demethylated metabolite (Monodes-IPU) have been detected after inoculation of isoproturon (soil "Feldkirchen" in Schroll and Kühn, 2004). Therefore further degradation of Monodes-IPU is most likely rate limiting for isoproturon mineralization by this microbial community. Mineralization results of the microbial community in liquid culture (e. g., appendix A figure A.1) show that this microbial community is able to completely mineralize isoproturon, but the metabolic pathway has not yet been identified.

After the "sink diffusion" experiment a wide variety of metabolites were detected in soil "Cambisol 3" outside the expanded clay particles. The number of metabolites actually is the

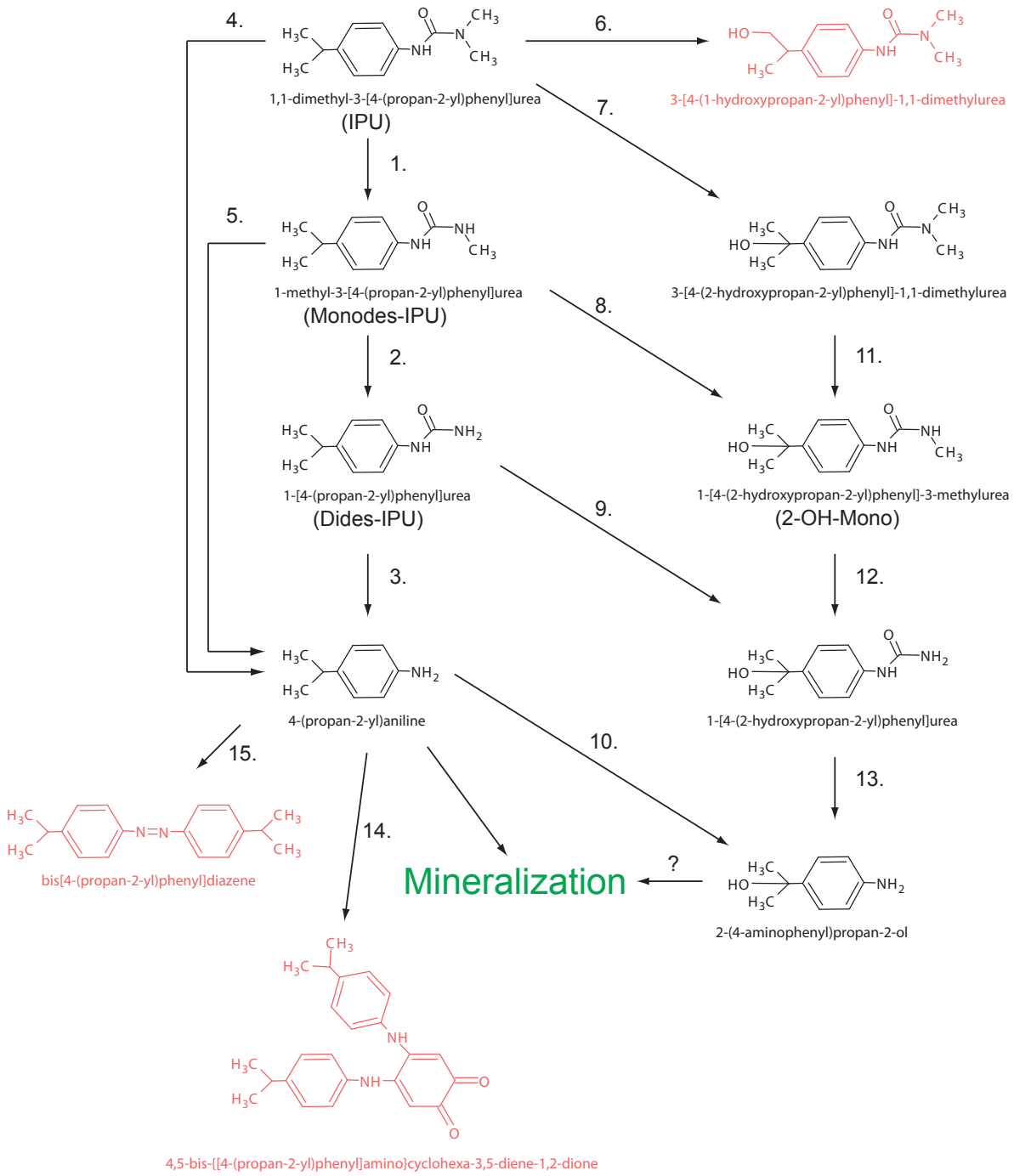


Figure 4.2: Degradation pathways of isoproturon in soil as proposed by Sørensen et al. (2003). Compounds depicted in red color are dead-end metabolites which can not be further degraded.

same as the number of those depicted in figure 4.2. So it is possible that all known isoproturon metabolites were present or that some other compounds with an intact phenyl group were produced. Although the parent compound and the monodemethylated metabolite were dominant, detection of numerous other metabolites suggests that the enzymes involved in the degradation of isoproturon did not act simultaneously and were produced by different organisms which can be expected for cometabolic degradation. The metabolite pattern did not change very much with distance to the expanded clay particle which indicates that the added microbial community had not colonized the soil outside the expanded clay particles.

4.3.4 Transport by diffusion

The experiment clearly showed that diffusion was the predominant process enabling contact between the microbial isoproturon degraders added to the soil on expanded clay particles. But diffusion was only sustained during the first half time of the experiment. Later most isoproturon derived ^{14}C was fixed as apparent bound residues and thus not available for diffusion anymore. This fixation seems to be primary a result of native soil microbial activity as the diffusion coefficient of isoproturon was nearly constant when microbial activity was inhibited. After the mineralization peak which indicates metabolic degradation was over cometabolic degradation was apparently dominant and diffusive transport probably negligible because only minor amounts of dissolved pesticide products remained. It follows that if a pesticide such as isoproturon which leads to the formation of considerable amounts of bound residues is applied to soil there is competition between metabolic degradation and (the undesired) transformation into bound residues. Both processes are sinks of soluble pesticide and metabolites.

Several studies have shown strong spatial variation of pesticide degradation and residue formation on the field (e. g., Walker et al., 2001), plot scale (e. g., Walker and Brown, 1983) and even millimeter (Gonod et al., 2003) scales. This variation is linked to the spatial variation of physical, chemical, and biological processes as well as variation of application (Price et al., 2009). Consequently microorganisms which metabolically degrade isoproturon can be expected to be distributed heterogeneously in soil. In fact the relatively short time after application when free isoproturon is available together with the relatively fast diffusion of isoproturon would favor a microbial strategy with disperse colonies which rely on diffusion for substrate supply.

In order to minimize formation of bound residues in soil fast metabolic degradation of the pesticide in soil is needed. This can only be achieved if the density of degrader cells is sufficiently high to allow much faster diffusion rates towards degradation sinks than rates of transformation to bound residues.

4.4 Conclusions and perspectives

At the micrometer to centimeter scale diffusion is an indispensable process for microbial activity in soils. Diffusion regulates the substrate supply of microorganisms and distributes DOM from preferential flow paths and organic matter conglomerations such as plant litter. The interaction of DOM diffusion with biological and physicochemical processes such as mineralization and sorption is difficult to investigate and consequently there is a lack of experimental results to validate and improve existing models.

A main part of this work was to investigate the intrinsic diffusion characteristics of DOM. It is of special interest if these characteristics promote fractionation of DOM or if such a fractionation could only result from interaction with the soil matrix. The appropriate hypothesis H_0 ("Fractions of DOM which show different stability against degradation in soil have different diffusion coefficients in free aqueous solution.") was formulated and an experiment conducted to test this hypothesis. Results for DOC, DON, and fractions distinguishable by fluorescence measurements do not allow to reject this hypothesis, but diffusion characteristics of soil DOM fractions were remarkably similar. Only the most labile fraction of fresh DOM extracted from barley straw exhibited a pronounced difference to other fractions in diffusion characteristics. Likely differences of intrinsic diffusion characteristics are not a reason of strong fractionation in soil. Different sorption characteristics and microbiological activity can be expected to be much stronger fractionating factors. The mean size of the DOM molecules as calculated from diffusion coefficients indicates that soil DOM contains at the most minor amounts of humic macromolecules. While this study provides valuable information about diffusion characteristics of soil DOM more experiments should be conducted to validate the results. Soil solutions of more soils should be analyzed for diffusion characteristics. Of particular interest would also be diffusion characteristics of fresh DOM from different sources, such as litter of different plant species or manure.

This study aimed to clarify important aspects of the role of diffusion in the development or reduction of heterogeneities in soil. Diffusion is driven by an increase of entropy and would

therefore be expected to reduce heterogeneities in soil. Two diffusion systems in soil, that are source induced and sink induced diffusion, were investigated. While diffusion from plant litter sources indeed was found to transport significant amounts of organic matter into the adjacent soil, the transported mass was small compared to the organic matter degraded directly at the source. The initial heterogeneity of organic matter added to soil created an analogous heterogeneity of microbial biomass and possibly microbial species. The opposite was true for sink induced diffusion. Here the heterogeneity of microbial species created heterogeneity of organic matter, in this case pesticide metabolites distribution. Most likely the microbial heterogeneity was preserved during the experiment as results indicate that the pesticide degraders were not able to colonize the soil.

The interaction of diffusion, short-term and long-term sorption processes, and microbial activity needs further investigation. Most important would be to study more soils to get statistically reliable data and to do time resolved experiments. Recently Poll et al. (2006, 2008) studied diffusion in soil using ^{13}C-labeled plant litter. Their experiments included enzymatic and phospholipid fatty acid analyses which allow to better include microbial activity into experimental evaluation. Such microbiological methods could be combined with ^{14}C-labeling, too. The experimental design in the study presented here allows a much better resolution of diffusion profiles because of the lower detection limit of ^{14}C measurements. If methods to investigate long term sorption processes, such as quantitative and qualitative measurements of bound residues, will also be included into future experiments it will become possible to improve existing models to a stage where accurate predictions of degradation and carbon sequestration processes could be achieved.

Although further research on diffusion of dissolved organic matter should be conducted, the experimental results of this work provide valuable data about diffusion characteristics of DOM and the interaction of diffusion with other processes in the context of microbial and organic matter heterogeneities in soil.

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Appendix A

Supplementary data

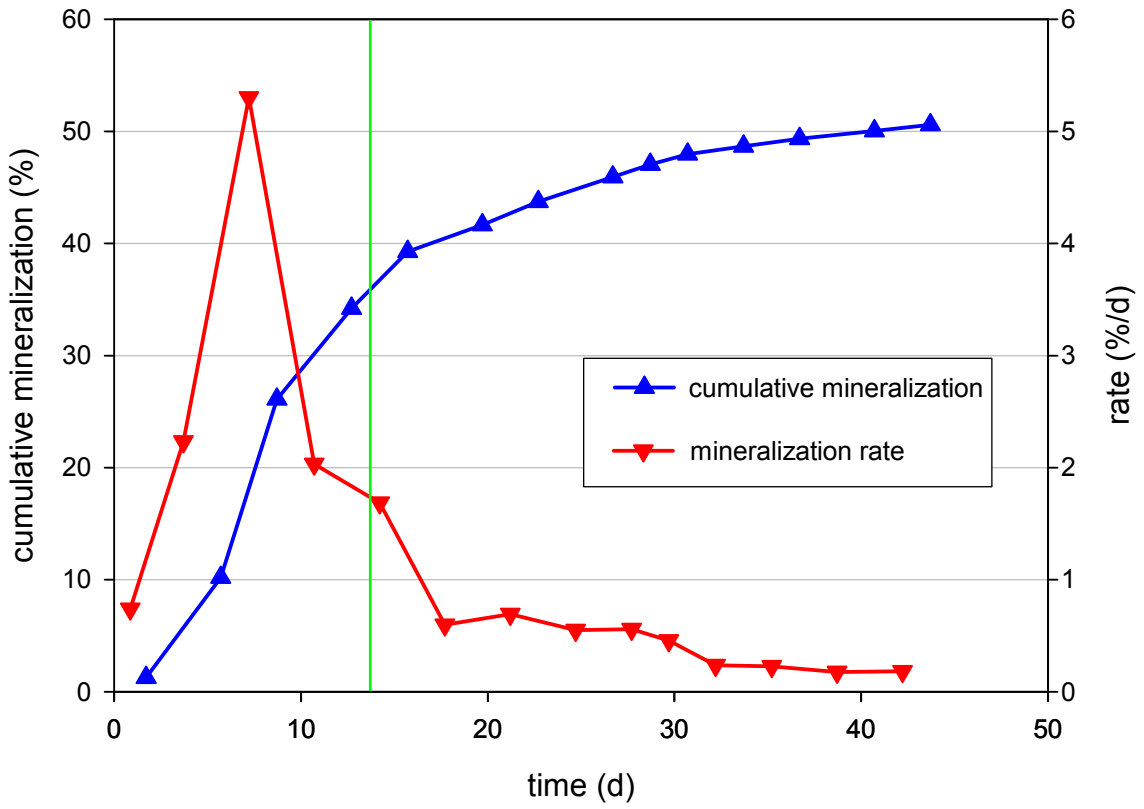

Figure A.1: Mineralization of isoproturon in liquid cultures. Unlabeled cultures were used to establish isoproturon degraders on expanded clay particles for the "sink diffusion" experiment (section 2.6.3). A parallel was run with ^{14}C-labeled isoproturon. Percent of applied ^{14}C released as CO_2 is depicted for this parallel as cumulative mineralization and as mineralization rate. The green line indicates when the expanded clay particles from the unlabeled cultures were transfered into soil to start the experiment.

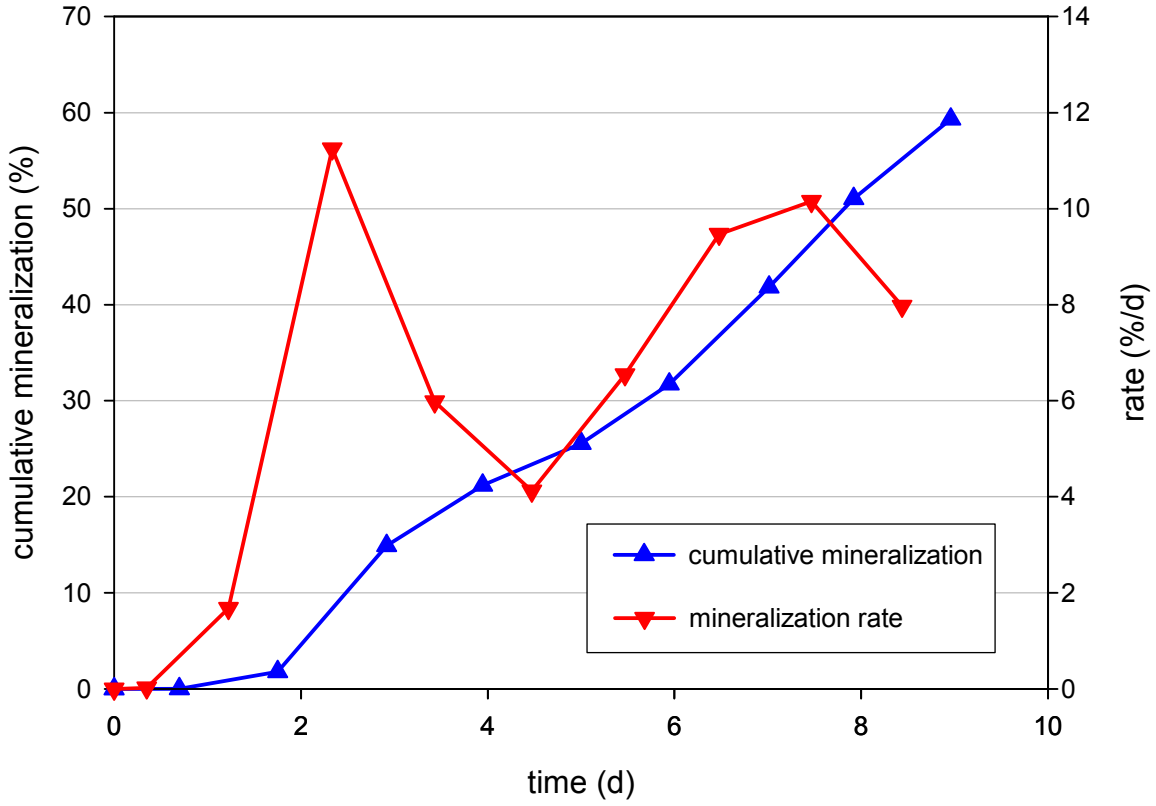

Figure A.2: Mineralization of ^{14}C-glucose in liquid cultures for ^{14}C-labeling of microbial biomass (section 2.3). Four replicates were connected in series for aeration. Percent of applied ^{14}C released as CO_2 is depicted as cumulative mineralization and as mineralization rate. Two peaks of the mineralization rate were observed. After 9 days the maximum of the second peak had been passed. The microbial biomass was harvested after 9 days and stored to be used later in the "source diffusion" experiment.

Table A.1: Dependence of C_{mic} on extraction ratio (section 2.8). Different extraction ratios were tested on 3 pre-incubated soils: "Cambisol 1", "Arenosol", and a cambisol from a site used for overwintering of cattle. The experiment was done in 4 replicates. According to Student-t-tests different extraction ratios did not result in significantly ($\alpha = 0.05$) differing C_{mic} values.

soil	extraction ratio	C_{mic} (mg carbon kg^{-1} soil)	standard deviation (mg carbon kg^{-1} soil)
"Cambisol 1"	1:5	249.5	29.1
	1:10	232.1	42.2
"Arenosol"	1:5	76.8	36.3
	1:9	51.0	59.8
cambisol (manure)	1:6	3318.7	6.4
	1:23	3297.2	22.9

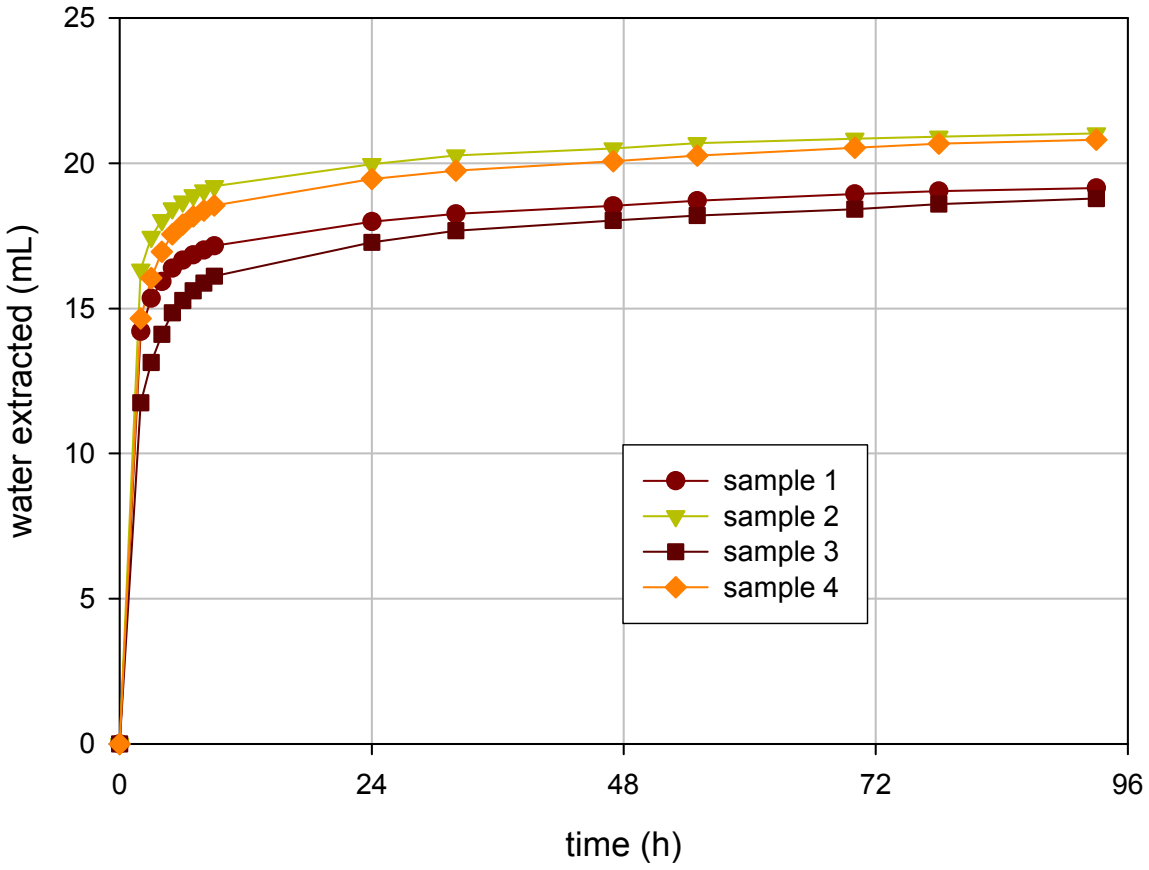

Figure A.3: Time dependence of extraction of soil solution by centrifugation. Undisturbed soil cores (80 cm^3) of soil "Cambisol 2" were saturated and then centrifuged at 4000 × g (section 2.9.2). The volume of solution released is depicted versus centrifugation time.

Table A.2: Properties of undisturbed "Cambisol 1" soil cores. Water content in the field, saturated and after centrifugation at 4000 × g are given as well as bulk density in the field. Furthermore analytical results of the extracted solutions are given.

replicate	water content field fresh ($g\,g^{-1}$)	water content saturated ($g\,g^{-1}$)	water content after centrifugation ($g\,g^{-1}$)	bulk density ($g\,cm^{-3}$)	TIC ($mg\,(C)\,L^{-1}$)	TC ($mg\,(C)\,L^{-1}$)	TOC ($mg\,(C)\,L^{-1}$)	TNb ($mg\,(N)\,L^{-1}$)
1	0.23	0.48	0.19	1.09	10.3	36.9	26.6	4.6
2	0.12	0.36	0.08	1.15	8.6	27.9	19.3	2.6
3	0.16	0.41	0.12	1.17	10.9	32.9	22.0	2.5
4	0.17	0.46	0.20	1.04	9.4	26.4	17.0	2.2
5	0.18	0.42	0.13	1.20	13.0	41.7	28.7	3.9
6	0.16	0.38	0.12	1.30	11.5	32.3	20.8	3.5
7	0.16	0.39	0.13	1.22	9.1	27.3	18.2	2.8
8	0.18	0.41	0.13	1.19	11.4	32.2	20.8	3.1
9	0.17	0.43	0.13	1.14	12.2	37.9	25.7	2.9
10	0.18	0.42	0.13	1.15	9.0	28.2	19.2	2.6
11	0.18	0.45	0.14	1.02	8.6	33.2	24.6	2.9
12	0.16	0.37	0.12	1.30	12.0	31.3	19.3	2.8
13	0.18	0.43	0.25	1.09	8.5	32.4	23.9	3.2
14	0.18	0.42	0.13	1.19	12.4	30.0	17.6	2.3
15	0.17	0.37	0.12	1.27	8.3	25.5	17.2	2.5
16	0.16	0.38	0.12	1.27	13.0	31.2	18.2	2.3
17	0.17	0.42	0.13	1.15	9.6	38.3	28.7	3.6
18	0.18	0.40	0.13	1.03	9.8	39.7	29.9	5.9
19	0.17	0.39	0.14	1.25	14.7	36.0	21.3	2.9
20	0.15	0.42	0.12	1.15	12.7	32.8	20.1	2.8
mean	**0.17**	**0.41**	**0.14**	**1.17**	**10.8**	**32.7**	**22.0**	**3.1**
standard dev.	0.02	0.03	0.04	0.09	1.9	4.6	4.1	0.9
standard error	0.00	0.01	0.01	0.02	0.4	1.0	0.9	0.2

Table A.3: Properties of undisturbed "Cambisol 2" soil cores. Water content in the field, saturated and after centrifugation at 4000 × g are given as well as bulk density in the field. Furthermore analytical results of the extracted solutions are given.

replicate	water content field fresh ($g\,g^{-1}$)	water content saturated ($g\,g^{-1}$)	water content after centrifugation ($g\,g^{-1}$)	bulk density ($g\,cm^{-3}$)	TIC ($mg\,(C)\,L^{-1}$)	TC ($mg\,(C)\,L^{-1}$)	TOC ($mg\,(C)\,L^{-1}$)	TNb ($mg\,(N)\,L^{-1}$)
1	0.25	0.31	0.18	1.36	0.6	33.6	33.0	10.6
2	0.22	0.26	0.17	1.52	1.1	21.7	20.6	14.3
3	0.25	0.35	0.18	1.12	0.7	12.6	11.9	15.0
4	0.24	0.33	0.17	1.23	0.9	15.0	14.1	14.7
5	0.28	0.34	0.17	1.27	n.d.	n.d.	n.d.	n.d.
6	0.22	0.32	0.18	1.30	0.6	14.8	14.2	4.5
7	0.24	0.32	0.18	1.25	0.8	11.6	10.8	8.8
8	0.24	0.35	0.16	1.08	0.7	9.3	8.6	11.3
9	0.23	0.33	0.18	1.17	0.2	26.5	26.3	15.2
10	0.23	0.30	0.20	1.28	0.6	18.9	18.3	17.2
11	0.23	0.32	0.18	1.23	0.4	13.8	13.4	20.0
12	0.24	0.33	0.18	1.18	0.7	16.8	16.1	21.1
13	0.24	0.31	0.19	1.31	0.5	19.7	19.2	20.3
14	0.25	0.32	0.20	1.25	0.7	14.8	14.1	14.0
15	0.23	0.30	0.19	1.22	1.0	15.5	14.5	18.7
16	0.25	0.30	0.19	1.33	0.8	17.1	16.3	22.5
17	0.25	0.30	0.17	1.39	0.7	13.3	12.6	16.8
18	0.25	0.31	0.18	1.33	0.5	15.3	14.8	16.8
19	0.24	0.32	0.17	1.34	0.2	13.6	13.4	12.7
20	0.23	0.29	0.15	1.31	0.4	13.9	13.5	19.9
mean	**0.24**	**0.32**	**0.18**	**1.27**	**0.6**	**16.7**	**16.1**	**15.5**
standard dev.	0.02	0.02	0.01	0.10	0.2	5.6	5.7	4.6
standard error	0.00	0.00	0.00	0.02	0.1	1.3	1.3	1.1

n.d.: not determined

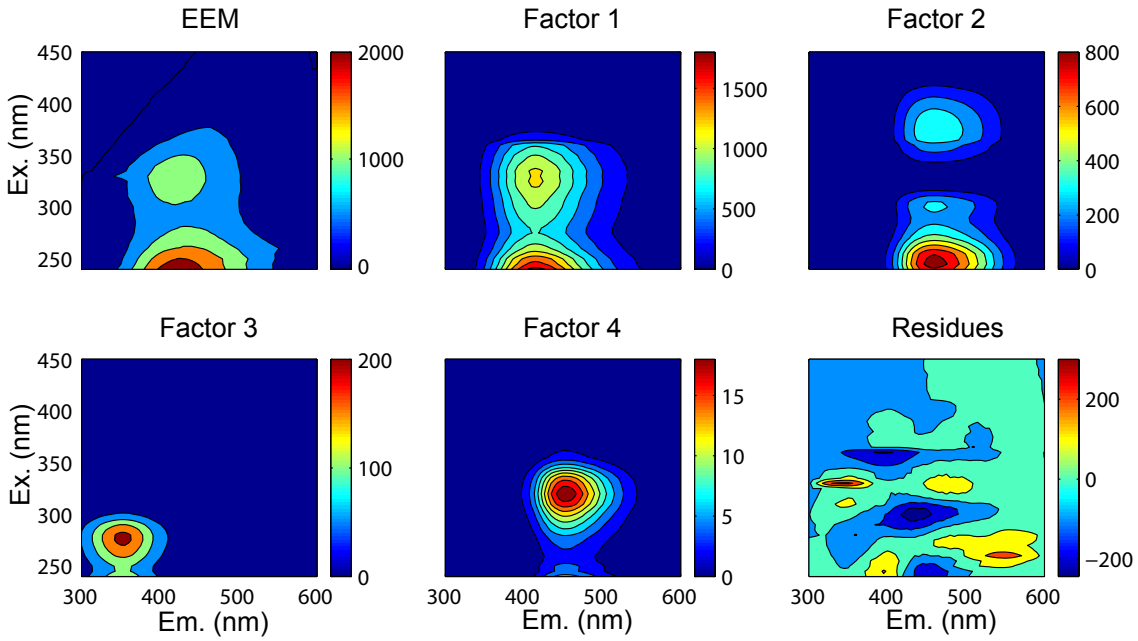

Figure A.4: PARAFAC result for "Cambisol 1" extract. The original spectrum, spectra of the fluorophore groups, and unexplained residues are shown.

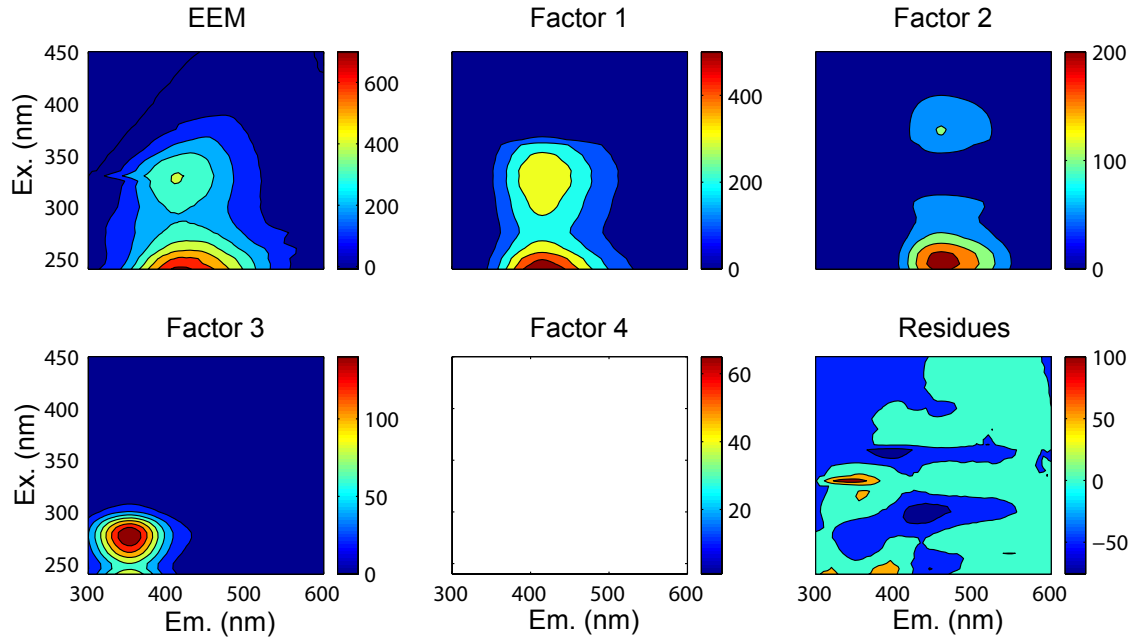

Figure A.5: PARAFAC result for "Cambisol 2" extract. The original spectrum, spectra of the fluorophore groups, and unexplained residues are shown. Factor 4 was below detection limit.

Appendix B

Calculations

Calculation of apparent isoproturon diffusion coefficient in soil "Cambisol 3"

$D_{aq} = 6 \cdot 10^{-6}\ \mathrm{cm^2\,s^{-1}}$

$\rho = 1.3\ \mathrm{g\,cm^{-3}}$

$d_s = 2.65\ \mathrm{g\,cm^{-3}}$ (Scheffer et al., 2002)

$\varepsilon = 1 - \frac{\rho}{d_s} = 0.51$

$m = 2$

$K_d = 0.54871\ \mathrm{cm^3\,g}$

$\theta = 0.12 \cdot 1.3 = 0.156$

$D_e = 6 \cdot 10^{-6} \cdot 0.51^2\ \mathrm{cm^2\,s^{-1}} = 1.6 \cdot 10^{-6}\ \mathrm{cm^2\,s^{-1}}$

$D_a = \frac{1.6 \cdot 10^{-6}}{1 + \frac{0.5487 \cdot 1.3}{0.156}}\ \mathrm{cm^2\,s^{-1}} = 2.8 \cdot 10^{-7}\ \mathrm{cm^2\,s^{-1}}$

Appendix C

Production of ^{14}C-labeled plant biomass

Figure C.1: Photograph depicting experimental setup for ^{14}C labeling of barley plants. The barley plants were grown in a desiccator under a light source (section 2.2). ^{14}C labeled CO_2 was produced continuously by dripping ^{14}C-carbonate into hot concentrated phosphoric acid.