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Biodegradation of the herbicide glyphosate in different agricultural soils

Nghia Nguyen Khoi

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´´Science cannot solve the ultimate mystery of nature. And that is because, in the last analysis, we ourselves are… part of the mystery that we are trying to solve"

Max Planck

(1858–1947)

Abstract

Glyphosate is the most widely used herbicide worldwide. Although glyphosate is believed to be a relatively safe compound because of its fast degradation, nowadays glyphosate and one of its principal metabolites (aminomethylphosphonicacid) are frequently detected in ground water. Inspite of the numerous studies concerning the effect of soil properties on adsorption/desorption of glyphosate in agricultural soils, studies regarding the effect of soil parameters on degradation of glyphosate in agricultural soils are still lacking and most of the studies in the literature are contradictory. The main reasons might be different experimental conditions focusing on few or one soil type. Therefore, a wide range of 21 different agricultural soil types were selected for this study.

The principal purposes of the study were

- to investigate the ability of 21 temperate soils (thirteen and eight agricultural soils from Germany and Slovenia, respectively) to mineralize glyphosate herbicide.

- to understand the processes and soil properties influencing the biodegradation, sorption, and desorption of glyphosate in 21 agricultural soils.

- to check the uptake and glyphosate mineralization abilities of extracted microbial cells in nutrient solution.

- to quantify the effect of glyphosate on soil microbial respiration in the various soils.

The experiments for biodegradation, *in situ* adsorption and soil respiration were conducted under test conditions: water tension of –15 kPa as soil moisture, a soil density of 1.3 g cm^{-3} and at 20 °C in the dark. Glyphosate used for all the experiments was ^{14}C-labelled. OECD and pore water extraction approaches were applied to determine adsorption and desorption behaviors of glyphosate in soils. Uptake and degradation of glyphosate by microbial cells was conducted in nutrient solution. In the course of the biodegradation $^{14}CO_2$, ^{14}C-extractable residues and ^{14}C-non-extractable residues were monitored. After the experiment the quantity of NaOH extractable residues in biodegradation experiments and glyphosate in nutrient solution were determined. The NaOH extractable residues were quantified by high-performance liquid chromatography (HPLC). Mass balance was calculated

at the end of biodegradation and liquid experiments. Bacterial cell counts were determined at the beginning and at the end of the biodegradation experiments.

The results from biodegradation experiments showed that the mineralization of glyphosate in the 21 different agricultural soils was very variable. Between 7.6 to 68.7 % of the applied ^{14}C-glyphosate was mineralized to $^{14}CO_2$ in the 21 different soils within 32 days of incubation. Moreover, the bioavailability plays an important role on degradation of glyphosate in soil. Glyphosate is rapidly taken up by the microorganisms in the soil solution and the highest mineralization rate is reached shortly after application. The NaOH extractable residues at the end of the biodegradation experiments widely varied and were relatively high (23-91 % of initial glyphosate), whereas the bound residues of glyphosate in soils were relatively low (2.5-11.4 % of the initial glyphosate).

Regarding the effects of soil properties on the biodegradation of glyphosate in soils, the results showed that the mineralization of glyphosate in soils is individually regulated and correlated by exchangeable H^+, soil pH, oxalate extractable Al^{3+} and bacterial cell numbers at the end of the experiments. However, the interacting functions of the different soil parameters on mineralization which were calculated by multiple regression analysis showed that mineralization of glyphosate is governed by exchangeable H^+, Ca^{2+} and K_2O collectively.

When applying OECD guideline 106 approach, both adsorption and desorption of glyphosate in soils are individually influenced by exchangeable H^+ and soil pH. Additionally, the glyphosate adsorption is controlled by soil pH, C% and silt collectively, whereas glyphosate desorption is controlled by exchangeable H^+, soil pH and Mg^{2+}collectively. However, when applying the pore water extraction approach (PW) no correlation between glyphosate (PW) and soil properties was found. This was caused by not only an artifact effect of high concentration of applied NaN_3, but also by the role of soil microorganisms in competition with soils for adsorption of glyphosate.

The results of nutrient solution concerning uptake and degrading abilities of extracted microbial cells showed that bacteria could take up and degrade glyphosate in nutrient solution where there are no sorption sites for glyphosate. The mineralization of glyphosate in nutrient solution depended on bacterial cell numbers. A large amount of glyphosate that was taken up by microbial cells shortly after application was mineralized over a long term period.

The results from soil respiration showed that effect of glyphosate application rate (10 µg glyphosate g^{-1} soil) depends on the type of soils. Fifteen out of 21 soils were found to have no effect of glyphosate on soil respiration. Only 1 out of 21 soils showed a depressing effect whereas 5 out of 21 soils showed a stimulating effect of glyphosate on soil respiration.

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List of acronyms and abbreviations

a.i	active ingredient
Al^{+3}_{Ox}	oxalate extractable Al^{3+}
AMPA	aminomethylphosphonicacid
Bq	becquerel
CEC	cation exchange capacity
CFU	colony-forming unit
$CFU_{beginning}$	colony-forming unit at the beginning of the experiments
CFU_{end}	colony-forming unit at the end of the experiments
Ci	curie
C-P	carbon-phosphorous
cum.mim	cumulative mineralization
d	day
DNA	deoxyribonucleic acid
Gly.	glyphosate
$H^{+}_{Exc.}$	exchangeable H^{+}
HPLC	high performance liquid chromatography
HSD	Honestly Significant Difference
Kd	adsorption coefficient values of chemicals in soil
kPa	kilopascal
L/h	liter per hour
LB	lysogeny broth
min	minute
NER	non-extractable residues
$OECD_{ad}$	adsorption experiment with OECD approach
$OECD_{de}$	desorption experiment with OECD approach
PBS	phosphate buffered saline
pKa	the logarithmic measure of the acid dissociation constant
POEA	polyethoxylated tallow amine
PW	pore water

PW_{de}	desorption experiment with pore water extraction approach
rcf	relative centrifugal force
T_{end}	at the end of the experiment
TLC	thin layer chromatography

1. Introduction

Glyphosate is the most widely used herbicide worldwide. It was classified as an easily degradable herbicide in the past. Although glyphosate is believed to be a relatively safe compound, in our days reports can be found that glyphosate has negative effects on human health. In addition, nowadays glyphosate and one of its principal metabolites (aminomethylphosphonicacid) have frequently been detected in ground water (Laitinen et al., 2009). Therefore, it is very important to know much more about the fate and degradation behavior of this pesticide in the environment to support the degradation.

It is reported in the literature that the degradation of glyphosate is a co-metabolic degradation dynamic (Torstensson, 1985). This means that soil microbes do not use glyphosate as a nutrient for their growth or for biomass formation. It has been observed that the degradation of glyphosate in various soils is very different and although many studies on the degradation of this compound were already conducted, differences in the degradation of glyphosate between soils are definitely not well understood. These differences could be linked to the bioavailability of the compound and the microbial activity of soils. The degradation of glyphosate is described in the literature as a two component first order degradation kinetic. This means that two processes might overlap: A first and rapid degradation process is followed or accompanied by a second and slow degradation process. Glyphosate is known to adsorb quickly to soils and thus it can be speculated that the first phase (= rapid process) of glyphosate degradation is positively correlated to the initial *in situ* bioavailability in soils or in other words, negatively correlated to the sorption in soils. Further, it can be speculated that the second phase (= slow process) of glyphosate degradation is linked to desorption of the herbicide and to subsequent microbial activity in soils.

One of the key factors that control degradation of pesticides in soil is their bioavailability which is influenced by their sorption. Usually, the sorption behavior of pesticides is determined according to the OECD guideline 106 (OECD approach). Applying the OECD guideline means that sorption is determined under very artificial conditions because this guideline includes addition of water in excess and strong shaking, which can break soil aggregates. This can lead to a significant increase of the available soil surface area which increasingly interacts with pesticide molecules (Wauchope et al., 2002). Therefore, a centrifugation approach was considered as a suitable method to determine sorption and bioavailability of chemicals to attain a more realistic parameter for the evaluation of their

leaching and biodegradation behavior in soil. Based on these findings, a specific approach for determining the *in situ* bio-availability of organic chemicals in soils was developed (Folberth et al., 2009b).

Moreover, some of the results in the literature regarding the behavior of glyphosate in soils are diverse. One of the reasons for such findings might be the reduced amount of different soil types in most of the former studies. Therefore, for being able to generalize findings about (1) the *in situ* bio-availability (*in situ* approach), (2) degradation of glyphosate and (3) effect of glyphosate on soil microbes as well, a wide range of very different agricultural soil types was selected to study the behavior of this pesticide in this thesis. The selected test condition under which the experiments were conducted is a critical point in general. Therefore, for biodegradation, *in situ* sorption and desorption experiments in this thesis, a soil density of 1.3 g cm^{-3} and a water tension of -15 kPa were applied because these experimental conditions are closer to realistic conditions like they are present in natural soils than the experimental conditions of the OECD approach (Schroll et al., 2006).

2. Background

2.1. Use of glyphosate in weed control

2.1.1. Glyphosate mechanism of action

Glyphosate is one of the most widely used herbicides in all over the world (Laitinen, 2009) because it is a broad-spectrum, non-selective systemic herbicide that can be used to control most weeds, both annual and perennial plants under many varied situations such as agriculture, forestry, orchards, vineyards, industry, no-till cropping systems and domesticity (Monsanto, 1996; Baylis, 2000; Tu, 2001; Kogan et al., 2003).

Glyphosate is effective in controlling all plant types by being absorbed into the plant mainly through its foliage but also through soft stalk tissue. It is then translocated to growing points of the plant where it acts on various enzyme systems inhibiting aromatic amino acids: tyrosine, tryptophan and phenylalanine that are essential for protein formation and secondary products in susceptible plants (Monsanto, 1996). This pathway also works in higher plants and microorganisms but not in animals. However, glyphosate-containing products are acutely toxic to animals (Cox, 1995). Plants treated with glyphosate slowly die over a period of days or weeks, and because the chemical is transported throughout the plant, no part survives. Because of this behavior, it is only effective on actively growing plants and used as a post-emergence herbicide, not as a pre-emergence herbicide (USDA, 1997; Reddy et al., 2008).

2.1.2. Application of glyphosate in agriculture

In agriculture, generally glyphosate is used to control directly the annual and perennial weeds prior to sowing crops. The use of glyphosate in agriculture is increasing, particularly resulting from the application of genetically modified plant varieties which tolerate glyphosate (Giesy et al., 2000). It is chosen for early-season weed control before planting and after harvesting (Duke and Powles, 2008; Laitinen, 2009). In reduced tillage or no-till cultivations, glyphosate is used to prepare fields before planting, during crop development and post harvest (Schuette, 1998; Ratcliff et al., 2006; Moneke et al., 2010).

The time and the frequency of glyphosate application vary and depend on kind of weeds and should not be higher than the recommended dose. In US, the annual maximum rate for glyphosate is limited to no more than 6.7 kg a.i ha^{-1} for crops and no more than 8.9 kg a.i ha^{-1} for non-crop uses. In agricultural areas, the common application rate for glyphosate is between 0.8 to 4.2 kg a.i ha^{-1} (USDA, 1997; Giesy et al., 2000). The best time to spray

glyphosate is when weeds are growing with adequate moisture and daily temperatures are between 13 °C and 19 °C (Goodwin, 2010).

2.1.3. Consumption of glyphosate in agriculture

Glyphosate was initially introduced into the market in 1970s. After introducing into the market, it has been one of the most consumed herbicides in the world with dozens of products by many companies (Woodburn, 2000). The products of glyphosate are sold approximately US$ 1,200 million annually and represent about 60 % of global non-selective herbicides sales (Buffin and Jewell, 2001) and about 50 % of Monsanto's total agricultural sales (Buffin and Jewell, 2001). Global consumption of glyphosate was over 600,000 tons in 2007 and it is expected to increase by an annual growth rate of over 12 % (Baird, 2008). A success of introduction of transgenic, glyphosate-tolerant crops also has led to a tremendous consumption of glyphosate in all over the world since 1996 and in 2001 glyphosate was the most used herbicide in USA with more than 60 % by volume of herbicide used. Nowadays almost 90% transgenic crops are glyphosate tolerant. The hectares of genetically engineered herbicide-resistant crops have increased each year. In 2006 the total area was 83 million hectares (Gianessi, 2008; Duke and Powles, 2008).

In European countries, glyphosate is one of top used herbicides. More than 18.31 tons of active substance of glyphosate was consumed (>20 % of the total used active substances). The use of glyphosate increased to 129 % between 1991 and 1995. In German arable agriculture, glyphosate was the 3rd most extensively used pesticide active ingredient (Buffin and Jewell, 2001; Eurostat, 2007).

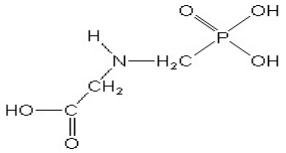
2.2. Properties, toxicology and occurrence of glyphosate in the environment

2.2.1. Chemical and physical properties of glyphosate

Glyphosate (*N*-(phosphonomethyl)glycine, $HOOC{-}CH_2{-}NH{-}CH_2{-}PO_3H_2$) is an organophosphorous herbicide that has a very stable carbon-phosphorous (C-P) bound in it's chemical molecule. It is an active ingredient in many herbicides with trade names as follows: Roundup, Roundup Ultra, Rodeo, Glycel, Ground Bio (Ermakova et al., 2010). Glyphosate is a multi-charged compound that has many functional groups that are positively (secondary amino group) or negatively (phosphonic and carboxylic group) charged in solutions (Jensen et al., 2009).

Glyphosate is an amphoteric and non-volatile compound, no photodegradation happens and it is stable in air. It is practically insoluble in most of organic solvents, for instance, ethanol, acetone and benzene because of its high polarity, but it is completely soluble in water (WHO, 1994; Giesy et al., 2000; Laitinen, 2009). It can have several pK_a values depending on soil pH (Figure 2.1). In soil, the distribution of glyphosate species depends on soil pH. There are several pKa values for acid dissociation constants (pK_{a1} = 2.22, pK_{a2} = 5.44 and pK_{a3} = 10.13). The charge of glyphosate in soil depends on soil pH. Glyphosate in low pH soils shows less negative charges as compared to the high pH soils (Borggaard and Gimsing, 2008). Some selected chemical and physical characteristics are presented in Table 2.1.

Table 2.1. Selected physical and chemical characteristics of glyphosate (WHO, 1994; Giesy et al., 2000 and Laitinen, 2009)

Parameter	Glyphosate
Chemical structure	
CAS number	1071-83-6 (acid)
Chemical name	[(N-phosphonomethyl)glycine)]
Empirical formula	$C_3H_8NO_5P$
Molar mass	169.08
Physical state and color	Crystalline powder, white
Melting point	200-230 °C
Specific gravity (density, 20 °C)	1.704
Henry's law constant	$< 7x10^{-11}$
Surface tension	0.072 N/m
K_{ow} LogP (pH 2-5, 20°C)	<-3.2
Water solubility (20°C)	11.6 g L^{-1}
Vapour pressure	$7.5x10^{-8}$ mm Hg
pKa	pKa_1 0.8, pKa_2 3.0, pKa_3 0.6 and pKa_4 10.0
Freundlich sorption coefficient (K_F)	0.6-303 L kg^{-1}
Photodegradation in soil	Not substantial over 31 days
Photodegradation in water	DT50 3-174 days
Half-life in water	DT50 5-91 days

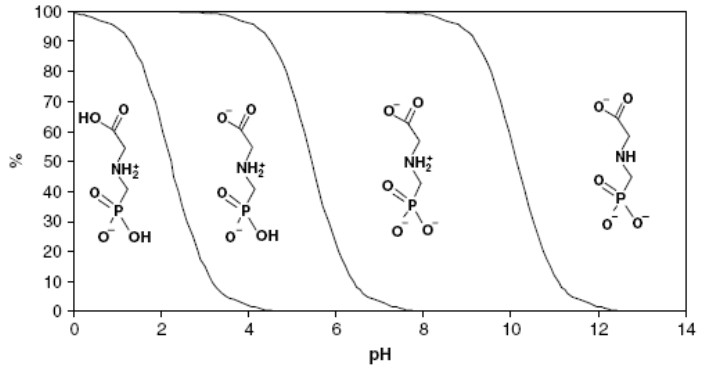


Figure 2.1. Distribution of glyphosate species in soil solution as a function of pH (Borggaard and Gimsing, 2008)

2.2.2. Toxicology of glyphosate

For human beings, glyphosate alone is not so toxic to human cell, but glyphosate formulations, e.g. Roundup is always more toxic than glyphosate (Richard et al., 2005). Using glyphosate-based herbicides was suspected to be a cause for pregnancy problems of some agricultural workers (Savitz et al., 1997). Glyphosate was shown to be toxic to endocrine and human placental JEG3 cells within 18 hours at infinitesimal concentrations at doses substantially lower than those found in agricultural use (200 μg mL^{-1}) (Richard et al., 2005). The indirect impact of glyphosate to human beings as carcinogenic and other pathological potentials was proven in human lymphocyte studies which showed that glyphosate can cause a change in the deoxyribonucleic acid (DNA) of sister chromatides (Watts, 2009a). In addition, in glyphosate formulations surfactants are used to improve solubility and penetration into plants; among these surfactants polyethoxylated tallow amine (POEA) is the predominant one. The glyphosate formulation in a combination with POEA has been shown to be very toxic for human peripheral blood mononuclear cells at a concentration of 56.4 μg mL^{-1} in laboratory experiments (Martinez et al., 2007; Benachour and Seralini, 2009). Other researches have shown that glyphosate concentration between 0.5 and 10 μg mL^{-1} affects human liver Hep G2 cells and causes an endocrine disruption of humans (Watts 2009b; Gasnier et al., 2009).

According to the producer Monsanto glyphosate is considered as a non-toxic compound for terrestrial and aquatic animals with a relatively low oral and dermal acute toxicity (USEPA, 1993; Buffin and Jewell, 2001), but glyphosate can be extremely toxic to non-target organisms beside the function to kill the target weeds or plants which has been verified by many studies (Busse et al., 2000; Tsui and Chu., 2003; Relyea 2005a; Relyea 2005b; Ratcliff et al., 2006; Lupwayi et al., 2009; Laitinen, 2009a; Vera et al., 2010). Reproductive effects of glyphosate were found that adverse dose-dependent effects happened on sperm quality and size of rabbits (WHO, 1994; Buffin and Jewell, 2001). Mutagenic effects of glyphosate and Roundup were recorded in a test with mice. A DNA damaging activity was observed in the mice's liver and kidney (Bolognesi et al., 1997). At a concentration of 10 μg mL^{-1}, glyphosate/Roudup produced genotoxic effects (DNA damage) on fish species *Prochilodus lineatus* in an exposure period of between 4 and 6 days (Langiano and Martinez, 2007; Cavas and Könen, 2007; Cavalcante et al., 2008).

There exits just little information about the biological effect of glyphosate on soil organisms. The direct toxicological effects of glyphosate or Roundup on soil organisms vary between organisms and species. Some soil invertebrates showed toxic effect of glyphosate, especially the springtail, *Onychiurus quadriocellatus* and beneficial predatory mite, *Amblyseius fallacies*. They showed their sensitivity of a decreased longevity when they were treated with glyphosate (Carlisle and Trevors, 1988). The toxicological effects of glyphosate on leaf litter invertebrates in the field were found when the sites were sprayed with a 1:10 v/v dilution of glyphosate-isopropyl 360 g active ingredient (a.i) L^{-1} (Lindsay and French, 2004). Increasing the application of glyphosate concentration was found to decline the numbers of soil fauna such as spiders, carabid beetles and bugs (Brust, 1990; Haughton et al., 2001). The nodule formation and root weight of *Rhizobium trifolii* was reduced at a glyphosate rate of 2 mg a.i kg^{-1} soil (Giesy et al., 2000).

Glyphosate directly and indirectly affects soil microorganisms. The directly toxic effect of glyphosate comes from an inhibition of amino acids synthesis across the shikimic acid pathway (Busse et al., 2000). Some beneficial microorganisms in soil are reduced by glyphosate and Roundup such as saprophytic, mycorrhizal fungi and nitrogen-fixing bacteria in soils after repeated application at a concentration of 1 μg g^{-1} of Roundup (Kremer and Means, 2009). Other studies also showed that glyphosate stimulated the growth of a number of fungal pathogens that cause diseases for many plants (Andrea et al., 2003; Watts 2009b). When treated with glyphosate and Roundup at a concentration of 9.2 mg kg^{-1}, soil fungal community structure was changed. Subsequently, soil respiration and rate of decay of organic matter were reduced (Levesque and Rahe, 1992; Abdel-Mallek et al., 2004; Vera et al. (2010).

2.2.3. Occurrence of glyphosate and its metabolites in surface water and ground water

Glyphosate is known as an immobile compound as it is strongly adsorbed in soil when it is applied. However, nowadays glyphosate and one of it's principal metabolites, aminomethylphosphonic acid (AMPA) have frequently been detected in surface water and even in ground water (Laitinen et al., 2009). In surface water of some European countries, glyphosate and AMPA concentrations were detected up to 1-6 μg L^{-1}, respectively, and such concentrations were higher than the European Union limit value for drinking water (Traas and Smit, 2003; Botta et al., 2009). The results from U.S Geological survey for nine states showed that AMPA was detected more frequently than glyphosate. Its concentration was similar or higher than that of glyphosate and both glyphosate and AMPA were detected more frequently

in surface water than in ground water as a result of runoff. In soils low concentrations of glyphosate and AMPA can persist for many years (Scribner et al., 2003; Scribner et al., 2007; Horth, 2010). In some special cases, glyphosate and AMPA can leach to ground water with a concentration up to 2.6 μg L^{-1} as a result of cracks in moraine clays and heavy rainfalls (GEUS, 2001). Glyphosate and AMPA were detected in drainage water from fields even 1 or 2 year after application, revealing that glyphosate and AMPA can be stayed longer within soil and gradually released over a long period of time (Brüsch, 2006; Kjaer et al., 2007; Schütte and Mertens, 2010). In the US and Canada, glyphosate and AMPA residues in water of lakes, ponds or streams were detected with a concentration between 5,153 and 35 μg L^{-1}, respectively (WHO, 2005).

Glyphosate in sediments and soils were between 0.5 and 5 mg kg^{-1}. The variation of the concentration of glyphosate in water, sediments and soils depends very much on the time of application and the rain events (Peruzzo et al., 2008).

2.3. Behaviour of glyphosate in soils

2.3.1. Degradation

Glyphosate degradation in soils is dominated by microbiological processes, which are mediated principally by bacteria and fungi (Rueppel et al., 1977; Giesy et al., 2000; Laitinen, 2009). There are two degradation pathways of glyphosate in soils. In the first degradation pathway, glyphosate-oxidoreductase enzyme catalyzes the cleavage of glyphosate to form glyoxylate and AMPA which later leads to the formation of water, carbon dioxide and phosphate (Sprankel et al., 1975a; Rueppel et al., 1977; Forlani et al., 1999; Giesy et al., 2000), whereas in the second pathway, C-P lyase enzyme from specific microorganisms, e.g. *Pseudomonas* (Shinabarger et al., 1984), *Agrobacterium* (Wackett et al., 1987), *Alcaligenes* (Talbot et al., 1984), and *Arthrobacter* (Pipke et al., 1987), respectively is involved to cleave the C-P bond of glyphosate with the formation of inorganic phosphate and sarcosine (Sviridov et al., 2011; Figure 2.2). The second degradation pathway occurs when phosphate is the limiting factor for the growth of microorganisms in soils (Dick and Quinn, 1995). Degradation of glyphosate greatly varies among soils, and the substance is rapidly degraded by a variety of soil microorganisms (Dick and Quinn, 1995; Wiren-Lehr et al., 1997; Forlani et al., 1999). The intensity of glyphosate degradation in soil depends on adsorption and desorption of glyphosate which control its bioavailability. The size and the activity of native glyphosate degrading microorganisms mainly regulate degradation of glyphosate (Sorensen et

al., 2006; Laitinen, 2009). In agriculture, the half life of glyphosate in soil varies in a time ranking between 1 and 197 days. In forestry soils, the DT_{50} ranged from 1 to 60 days (Wauchope et al., 1992). The degradation of glyphosate in soils seems to be a co-metabolic process since no lag phase is found under either aerobic or anaerobic conditions. Microorganisms use glyphosate as a sole source of phosphorous (Sprankle et al. 1975a; Forlani et al., 1999). Many studies have shown that the most common glyphosate degraders in soils are *Pseudomonas* spp. bacteria (Talbot et al., 1984; Kishore and Jocob, 1987; Jacob et al., 1988; Dick and Quinn, 1995; Moneke et al., 2010; Kolawole and Akinsoji, 2011). Beside *Pseudomonas spp.*, other species or genera also have a great contribution on the degradation of glyphosate in soil, for instance, *Rhizobiaceae* (Liu et al., 1991), *Arthrobacteria, Achromobacter* sp., (Pipke and Amrhein, 1988), *Candida kruseis* and *Yarrowia lipolytica* (Romero et al., 2004), *Acetobacter* sp. (Kolawole and Akinsoji, 2011), *Flavobacterium* sp. (Balthazor and Hallas, 1986), *Alcaligenes* sp. (Talbot et al., 1984; Lerbs et al., 1990), *Agrobacterium radiobacter* (Mcauliffe et al., 1990), and *Ochrobactrum anthropi* (Shushkova et al., 2010; Ermakova et al., 2010). Bacteria have the ability to take up glyphosate in liquid media when phosphate is depleted (Fitzgibbbon and Braymer, 1988; Pipke et al., 1987).

Many studies have focused on microbial degradation of glyphosate in soils and isolation of key degraders for glyphosate, but there exits just little information about uptake of glyphosate by microorganisms and there is still a lack of information regarding the uptake and mineralization rates of microbes shortly after application. This information is also important because soil microbes may compete with soil for glyphosate sorption. This issue should be clarified.

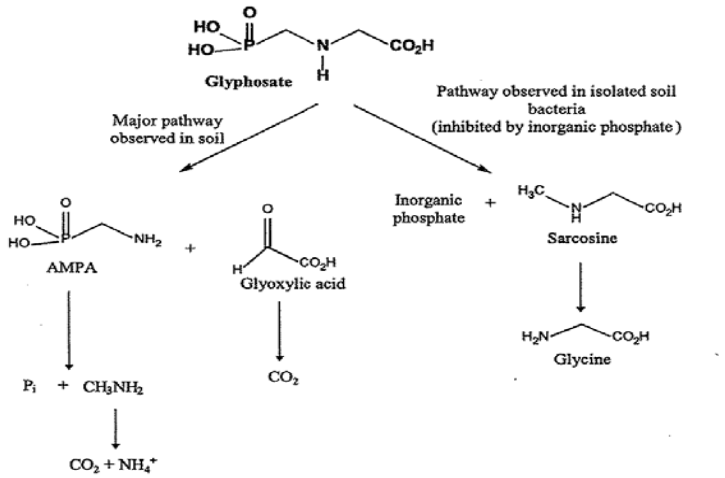


Figure 2.2. Degradation pathway of glyphosate in soils (Giesy et al., 2000)

The degradation of glyphosate in soil has been found by some earlier studies to correlate with some soil parameters. The summary for the literature review for the correlation between glyphosate degradation and soil properties is presented in Table 2.2.

Table 2.2. Literature review for the correlation between degradation of glyphosate and soil parameters

Parameters	Findings	Experimental conditions	References
With soil microorganisms	A positive correlation with the number of P*seudomonas* spp. (R^2 = 0.87)	8 different soil types; glyphosate concentration: 3.4 µg glyphosate g^{-1} soil; at the field moisture content; at 15 °C	Gimsing et al., 2004a
With microbial biomass and enzyme activity	A positive correlation with microbial biomass [y = 0.13x -12.33; R^2 = 0.84 where y is $^{14}CO_2$ in % of the initial ^{14}C after 26 days and x is microbial biomass in soil after 26 days (µg g^{-1} soil)]	9 different soil types; 2.5 kg a.i glyphosate ha^{-1}	Wiren-Lehr et al., 1997
	A positive correlation with enzyme activity (fluorescein diacetate hydrolytic activity; R^2 = 0.55)	4 different soil types; 1 µg glyphosate g^{-1} soil; 28 % water content; at 28 °C	Zablotowicz et al., 2009
With soil pH	A negative correlation with soil pH ($CaCl_2$) (R^2 = 0.53)	31 samples from 5 different soil types; 10 mM glyphosate; soil-water slurries; at 20 °C	Kools et al., 2005
With soil heavy metal content	A positive correlation with soil heavy metals: Cu_{total}, Pb_{total}, Zn_{total} and $Zn_{extractable}$ (R^2 = 0.40; 0.43; 0.37 and 0.61, respectively)	31 samples from 5 different soil types; 10 mM glyphosate; soil-water slurries; at 20 °C	Kools et al., 2005
	A negative correlation with $CaCl_2$ extractable Cu^{2+} [y = 4.61 – 0.095x; R^2 = 0.59 where y is the glyphosate mineralization after 80 days (%) and x is the $CaCl_2$ extractable Cu (mg Cu kg^{-1} soil)]	32 samples from 1 soil type; 10 mg glyphosate kg^{-1} soil; at 20 °C	Kim et al., 2011
With sorption coefficient (K_d) of glyphosate	A negative correlation with K_d of glyphosate (R^2 = 0.73)	4 different soil types; 1 µg glyphosate g^{-1} soil; 28 % water content; at 28 °C	Zablotowicz et al., 2009

The respective literatures which were cited in Table 2.2 were expected to have equation for regression, but unfortunately just a few authors gave the linear regression equation in their publications. Actually, most studies conducted the experiments only to check for dissipation, or disappearance of glyphosate in soils by using non-labeled glyphosate, therefore, information about the real degradation (mineralization) by soil microbes and pesticide bound residues in soils is still lacking. Moreover, from the literature, just a few publications have shown the correlation between real degradation of glyphosate in soils and soil parameters whereas most articles have presented the relationship between dissipation of glyphosate and soil parameters. Additionally, the amount of soil samples and different soil types for their experiments was really small, thus, they could not make any correlation between degradation of glyphosate and soil parameters, just speculated their interpretations. Besides, the experimental conditions varied to a great extent between the publications. Most experiments were conducted under conditions which are far away from real soil conditions, e.g. soil density and water content. All in all, in order to avoid the problematic issues presented above and to study the key soil parameters governing degradation of glyphosate, a large amount of soil samples with different soil types and the experimental conditions which are closer to the reality of natural soils should be considered.

2.3.2. Adsorption/Desorption

Degradation of pesticides in soils depends on adsorption and desorption capacity of the soils. Adsorption is one of the most important factors affecting the fate of pesticide in soil including leaching, volatilization, runoff and biodegradation (Kah and Brown, 2007). When applied to soil, pesticides tend to bind on soil particles by physical and chemical bonds. Adsorption mechanisms depend on pesticide and soil characteristics (Calvet, 1989). Several adsorption mechanisms are identified, e.g. hydrogen bindings, ion exchanges, interactions with metallic cations, polar interactions, charge transfers, London-Van der Waals dispersion forces and hydrophobic effects (Shoval and Yariv, 1979; McConnel and Hosserm, 1985; Miles and Moye, 1988; Calvet, 1989; Piccolo et al., 1992).

Glyphosate is strongly and rapidly adsorbed into soil matrix (Sprankle et al., 1975b). Glyphosate molecular structure has 3 different polar functional groups (carboxyl, amino and phosphonate groups) and especially the active phosphonate end group induces an inner sphere complex formation and covalent bonds between glyphosate and Al/Fe-oxides surfaces in soils (Sheals et al., 2002; Prata et al., 2003). Clay, clay mineral, iron and aluminum amorphous

hydroxides and organic matters are sites for adsorption of glyphosate in soil (Roy et al., 1989; Piccolo et al., 1994; Day et al., 1997; Rafiei Keshteli et al., 2011). The adsorption of glyphosate was ranked as follows: kaolinite < illite < montmorillonite < nontronite (Mc Connell and Hossner, 1985).

Soil pH is one of the most important soil constituents governing the adsorption of glyphosate. Glyphosate adsorption in soils will decrease when soil pH increases. This is explained by the fact that glyphosate molecules will produce more negative charges under high soil pH and simultaneously the negative charges of clay mineral, iron, and aluminum will increase too. Therefore, the glyphosate molecules can be repelled from the negative charge surfaces and adsorption will decrease (McConnel and Hosserm 1985). Conversely, according to Morillo et al. (2000) the adsorption of glyphosate on iron and aluminum oxides and hydroxides is high at intermediate pH and caused by ionic binding between the positive surface sites of minerals and the negative acid groups of glyphosate while the adsorption of glyphosate is much lower at very acid or very alkaline pH since oxides will have the same charge as glyphosate.

The mechanism of glyphosate sorption is similar to phosphate sorption on soil particles. Therefore, glyphosate and inorganic phosphates compete for the adsorption sites in soil (Hance, 1976; Rafiei Keshteli et al., 2011).

The reverse process of adsorption is desorption. Desorption of sorbed pesticide is also an important factor governing the mobility, bioavailability and biodegradability in soils (Zhang et al., 2004; Huang et al., 1998). It has been observed in soil and other natural sorbents that the sorption and desorption processes are not completely reversible (Longanathan, 2006). Desorption of glyphosate in soils has been shown to vary among soils. Desorbed amount from soils is low and depends on soil pH (Piccolo et al., 1994; Worrall et al., 2001; Al-Rajab et al., 2008; Jensen et al., 2009). Desorption of glyphosate was shown to be negatively correlated with the content of Al and Fe amorphous oxides in soils (Piccolo et al., 1994), but positively correlated with soil pH (Al-Rajab et al., 2008). Thus, the desorption amount of glyphosate in soils can vary between 5 and 97 % (Piccolo et al., 1994, Sorensen et al., 2006; Al-Rajab et al., 2008).

The effect of soil characteristics on adsorption/desorption is poorly understood although a lot of researches focusing on this area and the results from the literature are not very clear because most conclusions of studies were drawn without correlation analysis. The results sometimes conflict with each other, for example, the adsorption/desorption of glyphosate in soils were found to be related with organic matters (Yu and Zhou, 2005; Cruz et al., 2007; Rafiei Keshteli et al., 2011) and clay content (Glass, 1987; Wang et al., 2005), whereas organic matter and clay contents were found not to effect on adsorption of glyphosate (Autio et al., 2004; Gimsing et al., 2004b). One of the reasons for such findings might be that the number and choice of soil types which were used in the experiments were just a few. Moreover, the adsorption/desorption experiments were conducted using OECD approach. No method using soil pore water extraction for determining dissolution and adsorption of glyphosate in soils was found from the literature. The information regarding desorption and soil properties regulating desorption of glyphosate is really rare. In conclusion, in order to overcome the issues mentioned above and to study the key soil parameters governing adsorption/desorption of glyphosate, a large amount of soil samples with different soil types and the experimental conditions which are closer to the reality of natural soils should be considered. The summary for the literature review for the correlation between glyphosate adsorption and soil properties is presented in Table 2.3.

Table 2.3. Literature review for the correlation between adsorption of glyphosate and soil parameters

Parameters	Findings	References
With Al/Fe amorphous oxides	A positive correlation with iron oxides ($n = 16$; $R^2 = 0.67$)	Mamy and Barriuso, 2005
With organic matter	A positive correlation with organic matter in soil ($n = 6$; $R^2 = 0.86$)	Rafiei Keshteli et al., 2011
With phosphate	A negative correlation with unoccupied phosphate [1) $n = 9$; $R^2 = 0.72$; 2) $n = 5$; $R^2 = 0.99$; 3) $n = 16$; $R^2 = 0.82$; 4) $n = 4$; $R^2 = 0{,}98$]	1) Hance, 1976 2) Gimsing et al., 2004b 3) Mamy and Barriuso, 2005 4) Gimsing et al., 2007
With soil pH	A negative correlation with soil pH [1) $n = 5$; $y = -3.9x + 26.1$; $R^2 = 0.88$ where y is adsorption of glyphosate in soil (mmol glyphosate kg^{-1} soil) and x is pH_{CaCl2}; 2) $n = 16$; $R^2 = 0.87$]	1) Gimsing et al., 2004b 2) Mamy and Barriuso, 2005
With total Cu^{2+}	A positive correlation with total Cu^{2+} ($n = 16$; $R^2 = 0.89$)	Mamy and Barriuso, 2005

The respective literatures which were cited in the Table 2.3 were expected to have equation for regression, but unfortunately just a few authors gave the linear regression equation in their publications.

2.3.3. Runoff to surface water

Glyphosate easily moves to surface water by runoff (Botta et al., 2009). A part of applied glyphosate on the top of soil surfaces will dissolve in rain or irrigation water if irrigation or rain occurs after pesticide application. Subsequently, glyphosate penetrates into the soil. If the rain or the irrigation intensity is very large, the soil becomes saturated with water and subsequently, the runoff of rain or irrigation takes place (Luijendijk et al., 2003). Therefore, in the condition of wet soil surface, glyphosate is supposed to have a higher runoff rates as compared to the ´´dry´´ systems. The runoff of ´´dry´´ brick or soil system was about 1/3 of the runoff found in the ´´wet´´ system (Luijendijk et al., 2005). All in all, weather conditions before and after pesticide spraying are key factors controlling the extent of runoff of glyphosate, but the amount of ´´runoff-glyphosate´´ is strongly dependent on the time interval between application and rain (Luijendijk et al., 2005). Glyphosate concentrations in runoff water of up to 5 mg L^{-1} were found (Screpanti et al., 2005). Once glyphosate adsorbed to soil particles are washed by rain or irrigation water or blown by the wind into lakes or streams, the main amount of the glyphosate will stay adsorbed to the soil and settle to the bottom as sediment (Schuette, 1998).

2.3.4. Leaching to ground water

Sorption potential is the most important criteria for leaching potential of pesticides. Leaching process of pesticides in soils is an infiltration into soil profile. There is a strong relation between sorption, dissolution, degradation on one side and leaching of herbicides in soils on the other side. Leaching of pesticides can lead to a contamination of ground water which causes risk for non-target organisms and human´s health (Harrison, 1998). Pesticides with low water solubility have low leaching potential and they cannot easily move through soil profile to ground water as compared to pesticides with high water solubility. Pesticides with high sorption on soil particles thereby resist further infiltration through the soil profile (Tharp, 2012). Although glyphosate possesses some properties which make glyphosate strongly adsorbed into soil, a potential contamination of ground water by this herbicide can not be excluded (Landry et al., 2005; Vereecken, 2005). Concentration of glyphosate was higher than 0.1 g L^{-1} in shallow aquifers in Holland and other temperature regions (Candela et

al., 2010). The leaching of glyphosate depends on soil types. The leaching of glyphosate is reduced in soils which have high organic matter, clay contents, Al/Fe-oxides amounts, but low soil pH and phosphorous content (Torstensson et al., 2005; Kjaer el al., 2005; Candela et al., 2010; Laitinen, 2009b).

2.3.5. Formation of non-extractable (bound) residues

Non-extractable residues (NER) of organic compounds are defined as ´´bound residues 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 method must not substantially change the compounds themselves or the structure of the matrix" (Barriuso et al., 2008). The formation of non-extractable residues of glyphosate in soil has been shown to be various among soils (from 2 % up to 57 % of the initial glyphosate; Andrea et al., 2003; Getenga and Kengara, 2004; Weaver et al., 2007; Zablotowicz et al., 2009; Lancaster et al., 2010) and depends on soil properties such as soil texture, soil pH, organic matter and phosphate content and Al/Fe-oxides (Smith and Aubin, 1993; Mamy and Barriuso, 2005; Zablotowicz et al., 2009; Al-Rajab and Schiavon, 2010), but it is not dependent on soil organic matter (Zablotowicz et al., 2009).

3. Aim

The principal purpose of the study was to investigate the degradation and mineralization of the herbicide glyphosate in agricultural soils and to identify the soil parameters which control the mineralization of this herbicide. In order to overcome the problems mentioned above in **2.3.1** and **2.3.2** the experiments were conducted in a big variety of 21 different soil types to enable a representative applicability of the results. In addition, the selected experimental conditions (soil density: 1.3 g cm^{-1}, water content corresponding to pF 2.18) were as close as possible to realistic outdoor conditions to ensure realistic results

The specific objectives were:

(1) To study the innate ability of the agricultural soils to mineralize glyphosate

(2) To identify the key soil parameters regulating the degradation and mineralization, sorption and desorption of glyphosate in 21 agricultural soils

(3) To study the sorption and desorption of glyphosate in agricultural soils

(4) To compare the *in situ* and OECD approaches in determining sorption, desorption and bioavailability of glyphosate in soils

(5) To study the uptake and mineralization rates in a selected microbial community shortly after glyphosate application in nutrient solution

(6) To study the effects of glyphosate on soil microbial community using soil respiration as a key parameter

4. Materials and methods

4.1. Chemicals

^{14}C-glyphosate [N-(phosphonomethyl)glycine] was labeled on the phosphonomethyl group (PerkinElmer, Boston, USA) and had a specific radioactivity of 30 mCi mmol^{-1}. Since the radiochemical purity was < 80 %, ^{14}C-glyphosate was purified by thin layer chromatography to obtain a radiochemical purity of > 97.0 %. Thin layer chromatography (TLC) separation was performed on cellulose plates (20 x 20 cm, 0.1 mm, Merck, Darmstadt, Germany) using a solvent mixture of (parts by volume): Methanol/bidistilled water/NaCl 0,5M (180/60/0.3). Impure ^{14}C-glyphosate standard was dissolved in sterilized MQ water and this standard was applied on the cellulose plate at 1.5 cm position above the bottom of the plate; subsequently, the plate was put in a glass chamber containing the solvent mixture. After developing the plate in a time span of 4 hours, then the cellulose plate was taken out of the chamber and dried completely under the hood. Automatic TLC-Linear Analyzer (Tracemaster 20, Berthold, Wildbab, Germany) was used to detect the ^{14}C-labeled peaks on the cellulose plate. There were two peaks which consisted of glyphosate and AMPA. The R_f values of AMPA and glyphosate were 0.42 and 0.62, respectively. The peak areas of glyphosate and AMPA were labelled on the plate and scrapped separately. The scrapped cellulose powder containing ^{14}C-glyphosate was eluted with sterile MQ water through a glass filter (Filternutsche 50 ml, por. 4, Hattert, Germany). The purified standard was injected in ^{14}C-HPLC to prove the effectiveness of the purification. In general, a successive purification step is necessary to apply if still some ^{14}C-impurities can be quantified after the first purification step. Non-labeled glyphosate as well as the main degradation product aminomethylphosphonic acid (AMPA) and other metabolites (sarcosine, glycine, methylamine) were purchased from Dr. Ehrenstorfer (Augsburg, Germany), purity > 98.0 %.

Sodium hydroxide (NaOH), monopotassium phosphate (KH_2PO_4), sodium azide (NaN_3), sodium chloride (NaCl), calcium chloride dihydrate ($CaCl_2x2H_2O$), NH_4Cl, NH_4NO_3, methanol (CH_4O), diatomaceous earth, cellulose plates (20 x 20 cm, 0.1 mm), glyphosate column regenerant, water for chromatography, and all other chemicals for microbiological work (Agar, Yeast Extract, Pepton from Casein, and D(+)-Glucose-monohydrate) were purchased from Sigma-Aldrich (Steinheim, Germany). Scintillation cocktails (Ultima Gold XR, Ultima Flo AF, Permaflour E) and Carbosorb E were obtained from Packard (Dreieich, Germany).

4.2. Soils

4.2.1. Native sites of soils and soil sampling

Basis of soil properties, e.g. soil pH, amount of organic matter and soil textures, 21 agricultural soils were chosen in this study.

The 5 agricultural soils Feldkirchen (Fe), Hohenwart (Ho), Kelheim (Ke), Neumarkt (Ne) and Scheyern Lysi (Sc) were sampled at the lysimeter station of Helmholtz Zentrum München, Munich, Germany. These soils were originally collected as undisturbed soil cores from their respective native sites (Southern Germany). This research center is located on 50 hectare research campus in the North of Munich city, Germany (latitude: 48.250, longitude:11.567, elevation 472 meters, average annual temperature 7.5 °C, average annual rainfall 875 mm). The crop rotation was as follows: barley, corn, wheat (Grundmann et al., 2011).

The soils Ada-A02 (Ad), Berta-A02 (Be), Dunja-A06 (Du), Grace-A13 (Gr), Hanna-A15 (Ha), Lea-A18 (Le), Joy-A19 (Jo), Pear-A20 (Pe) were sampled from the research farm ´´Klostergut Scheyern´´ in Southern Germany located on tertiary hills, 40 kilometers north of Munich city (latitude: 48.257, longitude:11.221, 450–490 meters above sea level, a mean temperature of 8.7 °C and 803 mm $year^{-1}$ of rainfall). This experimental farm started in 1990 with a total of 153 hectares with 2 crop rotations. The valid crop rotations of chosen fields were winter wheat, potatoes, winter wheat, maize (Meyer-Aurich et al., 2001; Embacher et al., 2007).

The resting soils, collected from agricultural areas of northeastern part of Slovenia located in the Apace Valley, are named as follows Apace-njiva (Ap), Brezje (Br), Konjise (Ko), Lomanose (Lo), Lamanose (La), Skrinjar (Sk), Zepovci (Ze) and Zepovci (Plitv.) (ZeP). This valley is situated on the margin of Panonian Basin and covered up to 55 km^2 large area, 46.458 and 15.532 of latitude and longitude, respectively, 220 meters above sea level, average temperature of 10.34 °C and 794 mm $year^{-1}$ of rainfall. The crop rotations were maize, wheat and barley (Susnik et al., 2010).

The soils were sampled at a depth of 0-20 cm and air-dried in the lab for one week, crushed and sieved through a 2 mm sieve. The sieved soils were then packed in polyethene bags and stored in the freezer at -20 °C before use. Before freezing the actual water content of

the air-dried soils was determined. Therefore, the term ´´soil´´ will be used when considering as soil samples.

4.2.2. Soil characteristic analyses

The soil properties are shown in **Tables 4.1, 4.2** and **4.3**. The following soil parameters were analyzed at the soil laboratory, Center for Soil and Environmental Science, Biotechnical Faculty, University of Ljubljana, Slovenia. Soil texture was determined by using the sedimentation and pipetting method (Soil survey laboratory method manual, 1992). Soil pH was measured in soil extracts using 0.01 M $CaCl_2$ (SIST ISO 10390, 1996). Total carbon (organic carbon + carbonates) was analyzed by combusting soil samples at 900 °C and CO_2 was measured with Thermal Conductivity Detector analysis (SIST ISO 10694, 1996). Soil organic matter was determined according to the Walkely-Black method (SIST ISO 14235-Modification after Walkely-Black, 1998), and was calculated from the content of organic carbon using the conversion factor of 1.724. Total plant accessible potassium content (K_2O) in soils was analyzed by an extraction and spectrophotometry method (ÖNORML 1087-Modification: ammon-lactate, 1993). Total nitrogen in soils was measured by combusting the sample at 900 °C (SIST ISO 13878-Soil quality-Determination of total nitrogen content, 1998). Exchangeable calcium, magnesium, potassium and sodium in soil were determined by ammon-acetate extraction and atomic absorption spectroscopy method (Soil survey laboratory method manual, 1992). Exchangeable acidity (H^+) in soil was determined by Melichov method (extraction and titration-Modified after Peech, Soil survey laboratory method manual, 1992). Total CEC was performed as a sum of exchangeable calcium, magnesium, potassium, sodium and acidity (Cation exchange capacity-Soil survey laboratory method manual, 1992).

Total available phosphorous content (P_2O_5) in soil was determined according to the VDLUFA method (VDLUFA-method book, A6.2.1.1, 1991). Copper (Cu^{2+}) was analyzed by the CAT-element method (VDLUFA-method book, D 2.1 Finger test, CAT-Elements, A6.4.1, 1991). Oxalate-extractable iron and aluminum (Fe^{3+}_{Ox} and Al^{3+}_{Ox}) were determined by the VDLUFA method [VDLUFA MB I, D 2.1 (finger test), Schlichting/Flower, A2.4.3.1, 1991].

The optimal water content at a water potential at -15 kPa (pF 2.18) was measured in the Institute of Soil Ecology, Helmholtz Zentrum München, Research Center for Environmental Health, Munich, Germany by using a sand/kaolin box (Eijkelkamp, Netherlands). Air dried and sieved soil (< 2mm) was compacted into small metal rings with a volume of 9.4 cm^3 to

reach a soil density of 1.3 g cm^{-3} (Schroll et al., 2006). The pressed samples were placed on a plastic plate. The plate was then put in distilled water until the samples were saturated. They were then removed from the water and excess water was allowed to drain off for 15 minutes at laboratory temperature. The saturated samples were placed in a sand/kaolin box and equilibrated under a pressure of -15kPa. The mass of the samples was weighed periodically and when the mass was constant, the water content was determined and taken as the optimal water content (Kengara, 2010).

Bacterial cell counts were performed to count the cultivable heterotrophic bacteria in the different soils (Ngigi et al. 2011). Soil bacteria were extracted by mixing 1 g of soil (dry mass) with 99 mL of buffer solution containing (per L) 0.1 g NaCl, 0.02 g $CaCl_2 \cdot 2H_2O$, 0.2 g $MgSO_4 \cdot 7H_2O$, 5.0 g Tween 80. Prior to use the buffer solution was sterilized for 20 min at 121 °C in an autoclave machine. Buffer solution with soil was shaken vigorously for 1 hour on a shaker at 150 rpm. The soil particles were allowed to sediment for 10 min. Then 0.1 mL of the supernatant was transferred to 0.9 mL of sterilized buffer solution for further dilution steps. A total of 4 dilutions (10^{-1} to 10^{-4}) were established. Finally, 0.1 mL of each dilution was spread in triplicates on Lysogeny broth (LB) agar plate (10 g trypton enzymatic digest from casein, 5 g yeast extract, 5 g NaCl, 15 g agar and 0.1 mg cycloheximide in 1 L of distilled water). This medium was autoclaved for 20 min at 121 °C before use. The number of CFU was determined after three days of incubation at 25 °C by counting colonies.

Table 4.1. Physical characteristics of soil materials

Name of soil (site of origin)	Sand >0.05 mm [%]	Silt 0.05-0.02 mm [%]	Silt 0.02-0.002 mm [%]	Silt (0.002-0.05 mm) [%]	Clay (<0.002 mm) [%]	Water content at -15 kPa (%)
Ada-A02 (Ad)	62.5	11.4	16	27.4	10.1	21.9 (±0.8)
Apace-njiva (Ap)	66.4	15.9	15.3	31.2	2.4	20.7 (±1.0)
Berta-A02 (Be)	46.4	20	19.4	39.4	14.2	28.1 (±0.5)
Brezje (Br)	8.3	32.3	40.9	73.2	18.5	32.7 (±1.2)
Dunja-A06 (Du)	62.4	12.1	13.8	25.9	11.7	17.4 (±0.9)
Feldkirchen (Fe)	34.8	15.8	31.2	47	18.2	28.2 (±0.9)
Grace-A13 (Gr)	50.3	15.8	25.5	41.3	8.4	21.0 (±0.9)
Hanna-A15 (Ha)	62.3	10.2	14	24.2	13.5	18.4 (±0.9)
Hohenwart (Ho)	67.2	7.5	13	20.5	12.3	22.4 (±0.8)
Joy-A19 (Jo)	31.6	18.4	27.2	45.6	22.8	31.9 (±0.8)
Kelheim (Ke)	76.2	7.5	8	15.5	8.3	12.5 (±1.0)
Konjise (Ko)	33.8	30.1	30.1	60.2	6	34.6 (±0.7)
Lamanose (La)	10.3	25.3	44.3	69.6	20.1	35.8 (±0.9)
Lea-A18 (Le)	18.9	36	30.8	66.8	14.3	28.9 (±0.8)
Lomanose (Lo)	21.9	29.9	30.3	60.2	17.9	25.8 (±0.7)
Neumark (Ne)	85.5	3.4	5.4	8.8	5.7	12.6 (±0.8)
Pearl-A20 (Pe)	29.3	24.4	27.4	51.8	18.9	28.3 (±1.1)
Scheyern Lysi (Sc)	17.2	28.9	33.7	62.6	20.2	30.1 (±1.2)
Skrinjar (Sk)	67.5	10.6	16.4	27	5.5	19.2 (±0.8)
Zepovci (Ze)	41.3	16.4	26.7	43.1	15.6	24.0 (±0.9)
Zepovci (Plitv.) (ZeP)	11.8	37.5	34.7	72.2	16	27.4 (±0.7)

Table 4.2. Chemical characteristics of soil materials

Name of soil (site of origin)	pH ($CaCl_2$)	Organic mattter [%]	C [%]	N [%]	C/N	P_2O_5 (mg $100g^{-1}$)	K_2O (mg $100g^{-1}$)	Al_{Ox} (mg $100g^{-1}$)	Fe_{Ox} (mg $100g^{-1}$)
Ada-A02 (Ad)	5.7	2.9	1.7	0.2	8.4	17	4.1	63 (±0.4)	198 (±2.1)
Apace-njiva (Ap)	7.0	2.6	1.5	0.2	10.1	4	22.6	62 (±0.4)	248 (±3.5)
Berta-A02 (Be)	5.7	2.5	1.5	0.2	8.5	8	12.5	76 (±0.7)	265 (±2.8)
Brezje (Br)	5.2	2.8	1.6	0.2	8.1	5	21.1	187 (±2.1)	518 (±9.9)
Dunja-A06 (Du)	5.4	2.2	1.3	0.2	8.5	11	13.2	80 (±3.0)	211 (±7.8)
Feldkirchen (Fe)	7.0	3.4	2.0	0.3	7.3	39	9.4	139 (±1.4)	310 (±2.8)
Grace-A13 (Gr)	5.4	2.6	1.5	0.2	8.4	12	9.6	106 (±4.9)	259 (±12.0)
Hanna-A15 (Ha)	5.2	1.7	1.0	0.1	7.6	7	8.2	83 (±1.0)	215 (±2.8)
Hohenwart (Ho)	6.2	1.7	1.0	0.1	7.6	21	21.1	75 (±2.8)	206 (±8.5)
Joy-A19 (Jo)	5.9	2.7	1.6	0.2	8.2	34	43.2	101 (±3.0)	320 (±9.9)
Kelheim (Ke)	6.5	1.2	0.7	0.1	7.0	23	17	44 (±1.9)	132 (±2.1)
Konjise (Ko)	6.9	4.5	2.6	0.2	12.4	4	7.9	88 (±1.3)	381 (±7.8)
Lamanose (La)	5.8	4.3	2.5	0.3	8.3	5	18.7	134 (±0.0)	456 (±4.2)
Lea-A18 (Le)	5.2	1.9	1.1	0.2	7.3	6	23.8	107 (±3.0)	345 (±9.9)
Lomanose (Lo)	5.8	1.7	1.0	0.2	6.6	11	16.8	72 (±0.1)	252 (±2.1)
Neumark (Ne)	5.2	1.6	0.9	0.1	8.4	11	12.2	88 (±1.1)	110 (±1.4)
Pearl-A20 (Pe)	5.0	2.3	1.3	0.2	7.8	12	31.7	125 (±2.1)	319 (±3.5)
Scheyern Lysi (Sc)	5.5	2.7	1.6	0.2	9.2	20	5.3	102 (±0.7)	349 (±2.1)
Skrinjar (Sk)	7.1	1.6	0.9	0.1	7.1	21	24.7	57 (±0.1)	257 (±1.4)
Zepovci (Ze)	5.7	2.9	1.7	0.2	8.0	11	24.7	165 (±4.2)	476 (±12.0)
Zepovci (Plitv.) (ZeP)	5.2	1.9	1.1	0.2	7.3	8	20.2	147 (±1.4)	430 (±5.7)

Table 4.3. Chemical (cont.) and microbiological characteristics of soil materials

Name of soil (site of origin)	Ca	Mg	K	Na	H	CEC	Cu^{2+}	Bacterial cell counts
	[$mmol_c$ $100g^{-1}$]						(mg kg^{-1})	($x10^7$ CFU g^{-1})*
Ada-A02 (Ad)	8.48	0.78	1.00	0.04	5.70	16.0	4	0.3 (±0.1)
Apace-njiva (Ap)	11.05	2.34	0.10	0.04	1.45	15.0	4	0.5 (±0.1)
Berta-A02 (Be)	8.98	1.02	0.61	0.04	5.30	15.9	3	0.4 (±0.0)
Brezje (Br)	7.16	0.90	0.60	0.07	11.10	19.8	2	0.1 (±0.0)
Dunja-A06 (Du)	7.03	0.57	0.64	0.04	5.30	13.6	62	0.3 (±0.0)
Feldkirchen (Fe)	26.37	2.54	0.46	0.05	3.50	32.9	12	1.1 (±0.1)
Grace-A13 (Gr)	8.68	0.74	0.61	0.04	7.40	17.5	3	0.6 (±0.1)
Hanna-A15 (Ha)	7.18	0.49	0.21	0.04	5.65	13.6	2	0.5 (±0.0)
Hohenwart (Ho)	5.48	1.23	0.39	0.05	3.85	11.1	4	1.6 (±0.0)
Joy-A19 (Jo)	13.12	1.80	0.68	0.06	6.65	22.4	39	0.9 (±0.5)
Kelheim (Ke)	5.53	1.20	0.29	0.05	2.00	9.1	8	0.8 (±0.2)
Konjise (Ko)	10.77	4.64	0.10	0.06	3.15	18.8	7	0.1 (±0.0)
Lamanose (La)	16.41	3.57	0.32	0.06	9.20	29.6	4	0.4 (±0.1)
Lea-A18 (Le)	6.43	0.80	0.36	0.07	6.85	14.6	3	0.3 (±0.1)
Lomanose (Lo)	9.50	1.79	0.26	0.09	5.35	17.0	3	0.7 (±0.0)
Neumark (Ne)	2.56	0.36	0.23	0.05	4.30	7.5	1	0.7 (±0.1)
Pearl-A20 (Pe)	6.91	0.76	0.51	0.05	8.80	17.0	4	0.8 (±0.2)
Scheyern Lysi (Sc)	9.08	1.58	0.55	0.06	7.10	18.4	10	0.9 (±0.0)
Skrinjar (Sk)	10.80	0.46	0.13	0.06	1.50	13.0	4	0.5 (±0.0)
Zepovci (Ze)	7.75	0.67	0.89	0.05	10.55	20.0	4	0.3 (±0.1)
Zepovci (Plitv.) (ZeP)	4.39	0.50	0.71	0.10	9.35	15.1	2	0.1 (±0.0)

** CFU = colony-forming unit*

4.3. Glyphosate biodegradation experiments

4.3.1. Pesticide application procedure

Biodegradation of ^{14}C-glyphosate was studied in laboratory systems. All experiments were performed in 4 replicates with 50 g soil (dry mass) for each replicate. ^{14}C-glyphosate was dissolved in autoclaved and distilled water and mixed with non-labeled glyphosate which

was also dissolved in sterilized distilled water. This mixture was regarded as the application standard solution with a concentration of a.i of 5.42 µg μL^{-1} and a specific radioactivity of 166.70 Bq μg^{-1}. Prior to the experiments, the soils were equilibrated at 20 ± 1 °C room temperature for 2 weeks with a soil humidity of 75 % of the optimal water content at pF = -15kPa. The application standard (0.089 mL) was applied to an oven dried, pulverized soil aliquot of 3.5 g (dry mass) in a 50 mL glass beaker and carefully stirred for 1 minute with a spatula. After homogenous distribution the spiked aliquot was transferred to a 250 mL glass beaker containing the rest of equilibrated soils (46.5 g dry mass) and mixed for another 2 min. The total concentration of glyphosate was 10µg g^{-1} in each set corresponding to a total radioactivity of 83,000 Bq.

4.3.2. Test system, experimental conditions and samplings

After pesticide application, the soils were transferred to 500 mL brown incubation flasks, compacted to a soil density of 1.3 g cm^{-3} and soil water was adjusted to a water potential of -15 kPa. The flasks were covered with special rubber caps, and incubated at 20 ± 1°C in the dark for a period of 32 days. The soil humidity was controlled weekly and if necessary adjusted to a water potential of -15 kPa. The rubber caps were equipped with an air inlet and outlet system as well as a facility to trap the evolved CO_2. The air exchange system should prevent anaerobiosis in the incubation flasks and consisted of a canal which was made of a stainless needle with a diameter of 1 mm. To eliminate CO_2 from the ambient air entering the flasks, a 12 mL plastic syringe (Latex FREE, Tuttlingen, Germany) filled with granular CO_2 absorber (soda lime) was connected to the canal at the top of the cap. Below the cap a small plastic beaker was placed containing 0.1 M NaOH solution (10 mL) to capture $^{14}CO_2$ released from glyphosate mineralization from the soil samples. The NaOH solution was exchanged three times per week and from the collected solution an aliquot of 2 mL was mixed with 3 mL of scintillation cocktail Ultima Flo AF to determine $^{14}CO_2$ in a liquid scintillation counter (Tricarb 1900 TR, Packard, Dreieich, Germany).

At the end of the experiment, 30 g of each soil sample (dry mass) were used for soil pore water extraction, 7 g of each soil sample were extracted with NaOH to determine the quantity and quality of the extractable residues as well as to quantify the non extractable residues, while 1 g of each soil sample was used for bacterial cell counts.

4.3.3. Soil pore water extraction (PW approach)

To determine the pesticide residues in soil pore water the PW approach was used. At the end of the biodegradation experiments the soil was homogenized well and an aliquot of 30 g soil (dry mass) was transferred to a custom-built centrifuge container. This container includes an upper and lower part made of Teflon. The upper cup contains the soil sample and has a volume of 23.08 cm^3, which corresponds to a sample weight of 30 g at a soil density of 1.3 g cm^{-3}. In the lower part, soil PW is collected through a canal with a diameter of 2 mm, which connects these 2 parts. To avoid a clogging of the canal by soil particles, a glass frit with 120 µm average pore size was placed underneath the soil sample. The top of the upper part was closed tightly with an aluminum cap containing an O-ring (Folberth et al., 2009a). Centrifugation was carried out using Beckman J2-21 and Thermo Scientific RC 6+ centrifuges with Beckman JA-14 rotor (Beckman, Krefeld, Germany) and Thermo Scientific F14-6x250y rotor (Thermo Scientific, Langenselbold, Germany) at 21 °C for 90 minutes (min) with 9,170 relative centrifugal forces (rcf). After centrifugation, the extracted soil pore water was determined by weighing the lower cup including the extracted water and subtracting the mass of the lower cup. To quantify dissolved pesticide amount in soil solution, 0.1 mL of the extracted soil pore water were mixed with 5 mL of the scintillation cocktail Ultima Gold XR and measured in a Tricarb 1900 TR scintillation counter (Packard, Dreieich, Germany). As it was formerly proven (Folberth et al. 2009a) that the pesticide is equally distributed in the soil pore water of all soil pores, the total amount of dissolved pesticide was calculated from the total amount of water in the soil and from the concentration of glyphosate in the extracted pore water.

4.3.4. NaOH extraction, clean up and HPLC analysis

The extraction of glyphosate is not easy, especially when glyphosate has stayed in soils for long time. Former studies with a comparison of several solvents on glyphosate extraction in soils have shown that the most efficient solvent to extract glyphosate in soils was 0.1 M NaOH (Miles and Moye, 1988; Aubin and Smith, 1992). Thus, 0.1 M NaOH was applied as an extraction solvent to extract the glyphosate residues after biodegradation experiments. The NaOH extractable fraction is interpreted as adsorbed glyphosate to aluminum and iron oxides.

For NaOH extraction, the method used by Gimsing et al. (2004a) was applied. Seventh g of soil (dry mass) was extracted with 28 mL 0.1 M NaOH by shaking on overhead shaker (Reax 2, Heidolph, Schwabach, Germany) for 17 hours. The supernatant was collected after

centrifuging for 10 min at 3020 rcf. The supernatant was filtered through filter paper circles (No. 589/1, Whatman, Dassel, Germany). Radioactivity of the filtered supernatant was measured by scintillation counting using 100 µl of supernatant aliquot and 5 mL of scintillation cocktail Ultima Gold XR to quantify the NaOH extractable pesticide residues. Subsequently, extracts were concentrated and cleaned up before injecting to HPLC.

The concentration of the NaOH extracts was carried out on a Büchi Rotavapor R-114 which was connected to a Büchi water bath B-480 (Büchi, Flawil, Switzerland) at 30 °C to around 150 µl. The concentrated samples were filtered through centrifugal filters (modified Nylon 0.2 µm, 500 µl, VWR International GmbH, Darmstadt, Germany) in a table centrifuge (Biofuge Pico, Heraeus Instruments, Osterode, Germany) for 5 minutes at 9070 rcf. Purified samples were stored at -20 °C prior to HPLC analysis.

Twenty µl of each sample (NaOH extract) were injected via an Auto Sampler AS50 (Dionex, Idstein, Germany) to a HPLC system (GP50 Gradient Pump, Dionex, Idstein, Germany) that was connected with a Radioflow detector LB 509 (Berthold, Wildbad, Germany). The column, PRP-X400, 7µm, 4.6 x 250 mm (Hamilton, Reno, USA), was used at a flow velocity (isocratic) of 0.5 mL min^{-1}. 5mM KH_2PO_4 (pH 1.9) (A) and 5mM KOH (Regenerant-RG019) (Pickering Laboratories, Mountain View, CA 94043, U.S.A) (B) were used as mobile phases. The gradient program was: 1) 0-20 min: 100 % (A) and 0 % B; 2) 21-25 min: 0 % (A) and 100 % (B). ^{14}C-glyphosate and its metabolites (AMPA, sarcosine, glycine, methylamine) were identified by comparison of their retention times with standard substances. After each analysis the column was regenerated with Regenerant-RG019 (Pickering Laboratories, Mountain View, CA 94043, U.S.A) at a flow velocity of 0.5 mL min^{-1} for 30 min.

4.3.5. Quantification of non-extractable ^{14}C-labelled residues

After extraction with 0.1M NaOH, the rest of radioactivity remaining in the soil was considered as non-extractable residues. Soil material was intensively mixed and homogenized with 3.5 g diatomaceous earth (Sigma-Aldrich, Steinheim, Germany) for 2 min in a mortar. Four aliquots of each soil sample were weighed (aliquot mass varied between 0.1 and 0.3 g) in combustion cups and mixed with 8 drops of saturated aqueous sugar solution to accelerate and ensure a complete combustion of the ^{14}C. The combustion step was done with an automatic sample-oxidizer 306 (Packard, Dreieich, Germany). ^{14}CO2 from the combustion

was trapped in Carbo-Sorb E and mixed with Permaflour E before scintillation counting. In order to have a correct calculation for the extractable and non-extractable glyphosate residues after NaOH extraction, the proportion of extractable fraction which remained in the soil after centrifugation and filtration was calculated. This proportion was added to the extractable residues and subtracted from the radioactivity that was determined by combustion (non-extractable residues).

4.3.6. ^{14}C-mass balance

The advantage of using ^{14}C-labeled glyphosate for biodegradation studies is that it allows measuring directly the production of $^{14}CO_2$ and the formation of non-extractable residues which enables us to establish a ^{14}C mass balance. With help of the ^{14}C mass balance it is possible to quantify the different degradation and transformation paths of glyphosate in soils and to evaluate the quality of the practical handling during the experiments. Estimation of the mass balances of glyphosate in soils was performed at the end of the biodegradation experiments by calculating the sum of evolved $^{14}CO_2$, NaOH extractable pools and non-extractable residues.

4.4. Quantification of glyphosate in soil pore solution shortly after application

To compare the mineralization rate of glyphosate per cell in soil and in nutrient solution and to study the dynamics of ´´real bioavailability´´ and dissolution of glyphosate in soil solution shortly after application and to clarify the question, whether the microorganisms can influence the sorption process of glyphosate in soil, the following experiment was designed: ^{14}C-glyphosate biomineralization was monitored in biodegradation experiments in short time intervals in both conditions with and without NaN_3, at each sampling time intervals the dissolution of glyphosate in soil pore water using PW approach was measured.

4.4.1. Glyphosate in soil pore solution under biotic conditions

Biodegradation experiments were conducted in a continuously aerated laboratory system as adapted from Schroll et al. (2004) (Figure 4.1). The experiments were carried out with 30 g soil (dry weight) in 100 mL round biometer flasks with 24 sets. A ^{14}C-glyphosate standard solution with a concentration of 9.10 µg µL^{-1} and a specific radioactivity of 11.11 Bq µg^{-1} was used. The total concentration of glyphosate was 10µg g^{-1} in each set corresponding to a total radioactivity of 3,333 Bq (refer **4.3.1** and **4.3.2** for pesticide application procedure and experimental conditions). The incubation vessels were connected to a trapping system

and placed in the dark at 20 ± 1 °C for a period of 3 days. The vessels were aerated continuously at an air exchange rate of 0.5 liter per hour (L/h). After passing through the incubation flasks, the air was trapped in 2 subsequent wash bottles, which were filled with 10 mL 0.1 M NaOH solution to fix $^{14}CO_2$ from mineralization processes of glyphosate from soils. After 0.17, 0.33, 0.67, 1, 2, 3 days the NaOH solution was sampled and replaced by the same amount of fresh 0.1 M NaOH. At each sampling interval, two 2 mL aliquots of collected NaOH solution were mixed with 3 mL of scintillation cocktail Ultima Flo AF to determine $^{14}CO_2$ via liquid scintillation counting.

Four replicates of soil samples were sampled after 0.17, 0.33, 0.67, 1, 2, 3 days, respectively to determine dissolved amount of glyphosate in soil pore water by centrifugation (**4.3.3**). Cell counting (CFU) was determined at days 1, 2 and 3 hours of the experiment **(4.2.2**).

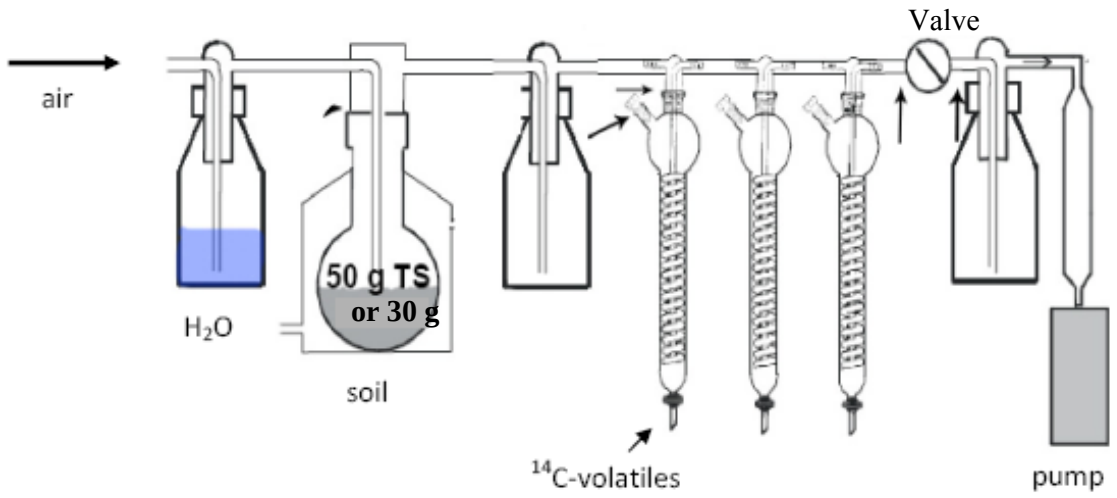


Figure 4.1. Continuously aerated biodegradation system

4.4.2. Glyphosate in soil pore solution under abiotic conditions

In a parallel experiment the amount of dissolved glyphosate in soil solution was determined under abiotic conditions. For this purpose the soil samples were sterilized as described in **4.7.1** (PW approach). The same ^{14}C-glyphosate application standard and application procedure were used as for the experiments to measure the glyphosate concentration in soil pore water under aerobic conditions (**4.4.1)**. Prior to the experiments, the soils were equilibrated at 20 ± 1 °C room temperature for 2 weeks with a soil humidity of 75 % of the optimal water content at pF = -15kPa. Glyphosate was applied on 30 g (dry weight)

sterilized soil of 24 experimental sets. After application of glyphosate, the sterilized soil samples were treated in the same way as the non-sterilized soil samples (**4.4.1**): The soils were transferred to 100 mL incubation flasks, compacted to a soil density of 1.3 g cm^{-3} and soil water was adjusted to a water potential of -15 kPa. The incubation vessels were placed in the dark at 20 ± 1 °C and aerated continuously on a closed laboratory trapping system (Figure 4.1) at an air exchange rate of 0.5 L/h for a period of 3 days. Four replicates of soil samples were taken 0.17, 0.33, 0.67, 1, 2, 3 days, respectively and soil pore water extraction was carried out as described in **4.3.3** to determine concentration of glyphosate in soil pore solution.

4.5. Glyphosate biomineralization capacity of one soil microbial community in nutrient solution

The result of the biodegradation experiments show that high amount of glyphosate was initially mineralized on the first day for all 21 soils (**5.1.1**). This means that bioavailability of glyphosate is very high shortly after application and that glyphosate mineralization in soils is mainly regulated by its bioavailability. Therefore, this experiment was conducted to measure the maximum biomineralization capacity of a soil microbial community shortly after application in an environment where the microbial activity is not limited by sorption processes to check the above mentioned hypothesis: ´´with a relatively similar microbial community, the mineralization of glyphosate in nutrient solution will be higher than that in soil since in nutrient solution, glyphosate is free for degradation by microbes and not limited by sorption processes´´.

4.5.1. Extraction of microorganisms from soil Feldkirchen

To extract microorganisms from soil, one gram of soil (dry mass) was given in 99.0 mL extraction solution (including per 1 L distilled water 0.1 g NaCl; 0.02 g $CaCl_2 \cdot 2H_2O$; 0.2 g Mg $SO_4 \cdot 7H_2O$ and 5.0 g Tween 80) and shaken vigorously in a shaker (3005 analogue orbital shaker, W x D x H: 380 x 510 x 140 mm, GFL, Burgwedel, Germany) at 150 rpm for 1 hour. The extraction solution was sterilized for 20 minutes at 121 °C in an autoclave machine. After extraction procedure the soil particles were allowed to deposit for 10 min. Approximately 80 mL of the supernatant containing soil extracted microbes and extraction solution was collected in 2 Falcon tubes (BD Falcon, Erembodegem, Belgium) and centrifuged in a Thermo Scientific RC6+ centrifuge with Thermo Scientific HS-3000 rotor at 3020 rcf for 10 min at 21 °C. After centrifugation, the supernatant was discarded. The remaining microbial

pellet was washed with 1*PBS (Phosphate Buffered Saline) to remove the carbon sources of the extraction solution by suspending the pellet with 20 mL of 1*PBS. The suspended pellet was centrifuged and the supernatant was discarded. The washing step was repeated for 3 times in total. After finishing the washing steps, a volume of 1 mL 1*PBS solution was added to 50 mL Falcon tube containing the microbial pellet to become a microbial suspension. This suspension was then mixed well. All microbial suspensions from 10 replicates were finally combined in a 50 mL Falcon tube resulting in a total volume of the combined microbial suspension of 20 mL. Additionally, each of the individual falcon tubes was rinsed with 0.5 mL of 1*PBS and these rinsing solutions were added to the 50 mL falcon tube that already contained the 20 mL combined microbial suspension. Finally, 30 mL microbial suspension in total was obtained in a 50 mL Falcon tube.

For bacterial cell counts the microbial suspension was homogenized well and 6 serial dilution steps were carried out by diluting 0.1 mL of the microbial suspension in 0.9 mL of sterilized buffer solution (**4.2.2**). Finally, 0.1 mL of 4 different dilutions (10^{-3} to 10^{-6}) was spread in triplicates on LB agar plate (**4.2.2**). The number of CFU was determined after three days of incubation at 25 °C by counting colonies.

*Preparation of 1*PBS solution:* 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, and 0.24 g KH_2PO_4 were dissolved in 800mL distilled H_2O, adjusted to pH 7.4 with 1N NaOH, then filled up to 1L in total with additional distilled H_2O. Finally, the solution was sterilized by autoclaving for 20 minutes at 121 °C.

4.5.2. Nutrient solution experiment

For the nutrient solution experiments the following mineral salt medium was used: 2.0 g NH_4Cl, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.5 g K_2SO_4, 2.5 mg $FeSO_4 \cdot 7H_2O$, 10.0 mg $CaCl_2 \cdot 6H_2O$, 2.0 mg $CuSO_4 \cdot 5H_2O$, 0.06 mg H_3BO_3, 20.0 mg $ZnSO_4 \cdot 7H_2O$, 1.0 mg $MnSO_4 \cdot H_2O$, 0.05 mg $NiCl_2 \cdot 6H_2O$; 0.3 mg $Na_2MoO_4 \cdot 2\ H_2O$ per 1 L of distilled water (Shushkova et al., 2009). The medium was sterilized by autoclaving for 20 minutes at 121 °C before use. To prevent the growth of fungi, cycloheximide was added to the media to yield 100 µg mL^{-1} total cycloheximide after the autoclaving.

A volume of 0.54, 1.35 and 4.86 mL microbial suspension were added to 100 mL incubation flasks containing 49.46, 48.65 and 45.14 mL of mineral salt medium, respectively

to result in 3 different cell numbers in nutrient solution as follows: 0.1 x 10^7 CFU 50 mL^{-1}, 0.3 x 10^7 CFU 50 mL^{-1} and 1.3 x 10^7 CFU 50 mL^{-1}, respectively. Each cell number treatment had 4 replicates. Finally, a volume of 0.048 mL of ^{14}C-glyphosate application standard having a concentration of 9.85 µg $µL^{-1}$ and a specific radioactivity of 1.69 Bq $µg^{-1}$ was applied to each 100 mL incubation flask to yield a concentration of 10 µg glyphosate mL^{-1} nutrient solution.

The samples were incubated on a shaker (3005 analogue orbital shaker, W x D x H: 380 x 510 x 140 mm, GFL, Burgwedel, Germany) at 120 rpm in the dark at 20 ± 1°C. To prevent the contamination of the sample in the incubation flask, filters (0.20 µm, Sartorius, Göttingen, Germany) were installed at the air in and outlet of the flasks (Figure 4.2). The flasks were aerated continuously on a closed laboratory trapping system. See **4.4.1** for description of the trap system and sampling of the trapping NaOH solution. The trapping solution was replaced by the same amount of fresh 0.1 M NaOH at days 1, 2, 3, 4, and 6. The collected NaOH (10 mL) was mixed with 10 mL of scintillation cocktail Ultima Flo AF to determine $^{14}CO_2$ by liquid scintillation counting. At days 1, 2, 3, 4, and 6 an aliquot of 0.1 mL nutrient solution was taken to perform cell counting by spreading serial dilutions on LB agar plates (**4.2.2**). At the end of the experiment, the remaining radioactivity in nutrient solution was determined by mixing 0.5 mL aliquot of the nutrient solution with 4.5 mL of scintillation cocktail Ultima Gold XR and measuring this sample in a liquid scintillation counter.

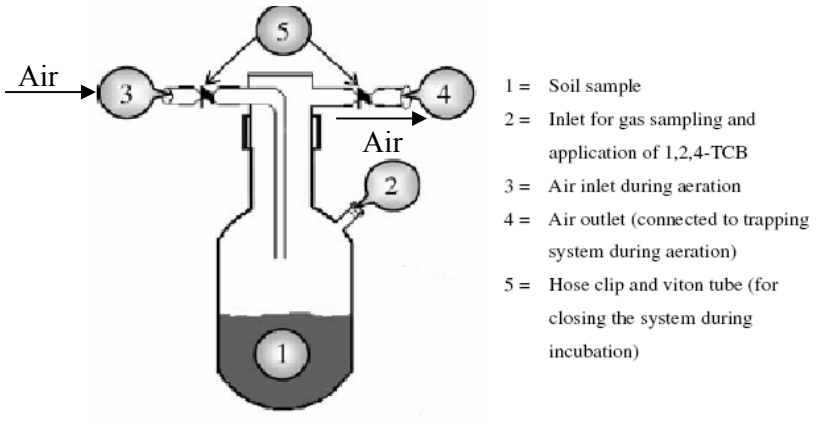


Figure 4.2. Test system used for nutrient solution incubation

4.6. Dynamics of glyphosate biomineralization and glyphosate uptake by soil microorganisms in nutrient solution

The results from biodegradation and desorption experiments show that the mineralized amount was higher than dissolved amount of glyphosate during 6 days (**5.1.1**). This is astonishing. A hypothesis arising is that soil bacteria can take up glyphosate in a large amount by soil bacteria shortly after application and this incorporated glyphosate is further mineralized to CO_2 with time. Therefore, an experiment was conducted to elucidate this process. The aim of this experiment was to investigate the dynamics of glyphosate biomineralization and glyphosate uptake by microbial cells in nutrient solution shortly after application and to clarify the question, whether the ^{14}C-glyphosate that is taken up by the microorganisms in the first nutrient solution is mineralized in the second nutrient solution. Thus, the following experiment was designed: ^{14}C-glyphosate biomineralization was monitored in nutrient solution (phase I) in short time intervals, the microbial biomass was harvested from the nutrient solution, transferred to a new nutrient solution (phase II) with non labeled glyphosate and the production of $^{14}CO_2$ was measured.

4.6.1. Glyphosate biomineralization in nutrient solution

The biomineralization experiments in nutrient solution were carried out with microorganisms extracted from soil Feldkirchen (**4.5.1**). After extraction, a total volume of 48 mL microbial suspension was obtained in a 50 mL Falcon tube.

The determination of the bacterial cell counts in the microbial suspension and the preparation of the mineral medium used for the nutrient solution are described in **4.5.1** and **4.5.2**, respectively.

A volume of 0.5 mL of microbial suspension was added to 100 mL incubation flasks containing 49.50 mL of mineral medium to result in a cell number of 3.5 x 10^6 CFU 50 mL^{-1} liquid medium. For glyphosate application, a volume of 0.058 mL ^{14}C-glyphosate application standard having a concentration of 8.72 µg glyphosate μL^{-1} and a specific radioactivity of 16.54 Bq μg^{-1} was applied to each 100 mL incubation flask in order to reach a final concentration of 10 µg herbicide glyphosate mL^{-1} in nutrient solution. The experiment was performed in replicates of twelve.

The samples were incubated on a shaker (3005 analogue orbital shaker, W x D x H: 380 x 510 x 140 mm, GFL, Burgwedel, Germany) at 120 rpm in the dark at 20 ± 1 °C. To prevent the contamination of the sample in the incubation flask, filters (0.20 µm, Sartorius, Göttingen, Germany) were installed at the air in and outlet of the flasks (Figure 4.2). The flasks were aerated continuously on a closed laboratory trapping system. See **4.4.1** for description of the trapping system and sampling of the trapping NaOH solution. The 0.1 M NaOH solution was exchanged after 0.17, 1, 2 and 3 days. The collected NaOH (10 mL) was mixed with 10 mL scintillation cocktail Ultima Flo AF to determine $^{14}CO_2$ by liquid scintillation counting.

4.6.2. Glyphosate uptake by microorganisms in nutrient solution

After 0.16, 1, 2 and 3 days incubation, four nutrient solution replicates were transferred to 50 mL sterilized Falcon tubes (BD Falcon, Erembodegem, Belgium) and the cells were harvested by centrifugation (Thermo Scientific centrifuge with Thermo Scientific HS-3000 rotor) at 3020 rcf for 10 min at 21 °C. After centrifugation the supernatant was collected and the microbial pellet was washed with 20 mL 1*PBS (**4.5.1**). This washing step was repeated 3 times and all the supernatants were collected. After finishing the washing procedure, all supernatants were combined, and then two 10 mL aliquots of supernatants were mixed with 10 mL scintillation cocktail Ultima Gold XR and measured in a scintillation counter to determine the radioactivity of the cell free nutrient solution.

The microbial pellet was suspended in 1 mL 1*PBS, mixed well and then transferred to a 100 mL incubation flask containing 49 mL of fresh mineral salt medium. Finally, 0.05 mL non-labeled glyphosate (10µg μL^{-1}) was applied to result in a final glyphosate concentration of 10 µg mL^{-1} nutrient solution. Two filters (0.20 µm, Sartorius, Göttingen, Germany) were installed at the air in and outlet of the flasks. The flasks were incubated on a shaker (3005 analogue orbital shaker, W x D x H: 380 x 510 x 140 mm, GFL, Burgwedel, Germany) and aerated continuously on a closed laboratory trapping system. Every 24 hours the NaOH trapping solution was sampled to measure the evolved $^{14}CO_2$. The whole experiment lasted for 22 days. After 22 days of the experimental course, the microbial biomass and the aqueous phase of the liquid culture were separated by centrifugation. After centrifugation, the microbial biomass was washed 3 times with 20 mL 1*PBS. All supernatants were collected to determine the radioactivity in cell free liquid phase as described above. The microbial biomass was transferred to combustion cups, and combusted in an automatic sample-oxidizer (**4.3.5**) to determine the radioactivity in the microbial biomass.

4.7. Glyphosate adsorption and desorption experiments

The adsorption and desorption behavior of glyphosate in the tested 21 soils was measured by using two approaches: OECD approach and PW approach. Normally, adsorption experiments are conducted according to the OECD Guideline 106 that is used to determine adsorption coefficient values of chemicals in soil (K_d) and is a conventional approach (OECD, 2000). Since the conditions of the OECD approach are rather artificial additionally the PW approach was used to measure adsorption and desorption behavior of glyphosate under more realistic conditions. All the experiments were conducted in 4 replicates.

4.7.1. Soil sterilization procedure with sodium azide (NaN_3)

In order to inhibit microbial glyphosate degradation during the sorption experiments the soils were sterilized with NaN_3 before application of glyphosate. Preliminary investigations were conducted to determine the appropriate NaN_3 concentration and the adequate equilibration time for a sufficient and sustainable inhibition of the microbial activity. According to the results of these investigations which are described in detail in **4.9**, the soil sterilization procedure was conducted as follows:

For OECD approach [7g (dry mass) total soil sample]: 0.14 mL NaN_3 solution with a concentration of 325 mg mL^{-1} was applied to 7 g (dry mass) soil in a 50 mL glass beaker to give a final NaN_3 concentration of 6,500 µg g $soil^{-1}$ and carefully stirred for 1 minute with a spatula. After homogenous distribution the soil was transferred to a 40 mL Teflon tube. Finally, the soils were compacted relatively to a soil density of 1.3 g cm^{-3} and soil water was adjusted to a water potential of -15 kPa. Subsequently, the Teflon tubes were covered with caps and placed in a desiccator containing water at the bottom to prevent the loss of water from the soil samples. After an equilibration period of 3 days, the soils were ready for the adsorption experiments.

For PW approach [30g (dry mass) total soil sample]: a mass of 195 mg NaN_3 were applied to an oven dried, pulverized soil aliquot of 3.5 g soil (dry mass) in a 50 mL glass beaker and carefully stirred for 1 minute with a spatula. After homogenous distribution the spiked aliquot was transferred to a 100 mL glass beaker containing the rest of the equilibrated soil (26.5 g dry mass). The soil was then stirred for another 2 min. The final NaN_3 concentration was 6,500 µg g $soil^{-1}$. Finally, the soils were compacted to a soil density of 1.3 g cm^{-3} and soil water was adjusted to 75 % of a water potential of -15 kPa. Subsequently, the

beakers were covered with Parafilm and equilibrated for 3 days in a desiccator containing water at the bottom.

4.7.2. Glyphosate adsorption experiments using OECD approach

A standard solution with a concentration of 0.987 µg μL^{-1} ^{14}C-glyphosate and a specific radioactivity of 24.39 Bq μg^{-1} was used. A volume of 0.061 mL of the application standard was applied to 7 g sterilized soil in a 40 mL Teflon tube in order to reach a final concentration of glyphosate in the soil of 10 µg g $soil^{-1}$. Furthermore, the rest of liquid phase (35 mL 0.01M $CaCl_2$ in total) was added to obtain the ratio mass between soil matrix and liquid phase of 1:5. The Teflon tubes were placed on a Reax 2 overhead shaker (Heidolph, Schwabach, Germany) and shaken for 16 hours under the laboratory temperature (20 ± 1 °C). In preliminary experiments (**4.10**) a shaking time of 16 hours was figured out as appropriate for reaching sorption equilibrium of glyphosate between soil and liquid phase. After shaking time, the soil samples were centrifuged at 20 ± 1 °C for 25 min at 35,000 rcf with Beckman J2-21 centrifuge and a Beckman JA-20 rotor (Beckman, Krefeld, Germany). The supernatant was filtered through filter paper circles (No. 589/1, Whatman, Dassel, Germany). The filtered supernatants were collected to measure dissolved amount of ^{14}C-residues in scintillation counter. For each sample, 10 mL of water phase was measured with 10 mL scintillation cocktail Ultima Gold XR. The soil samples after centrifugation were mixed well with 3.5 g diatomaceous earth and aliquots were taken to determine the residual radioactivity in the soil after combustion in an automatic sample-oxidizer (adsorbed amount of glyphosate) (**4.3.5**). In order to have a correct calculation for the dissolved and adsorbed glyphosate after adsorption experiment, the proportion of dissolvable fraction which remained in the soil after centrifugation and filtration was calculated. This proportion was added to the dissolvable amount and subtracted from the radioactivity that was determined by combustion (adsorbed residues).

4.7.3. Glyphosate desorption experiments using OECD approach

Like the sorption experiments, the soil for desorption experiments was sterilized with NaN_3 before application of glyphosate (**4.7.1**).

The same ^{14}C-glyphosate application standard and application procedure were used for both adsorption and desorption experiments (**4.7.2**). After application the Teflon tubes were placed on an overhead shaker and shaken for 16 hours under the laboratory temperature (20 ±

1 °C). This represented the initial adsorption step. After shaking time, the soil samples were centrifuged at 20 ± 1 °C for 25 min at 35,000 rcf with Beckman J2-21 centrifuge and a Beckman JA-20 rotor. After centrifugation, 25 mL of the supernatant was sampled and replaced with 25 mL of herbicide free 0.01 M $CaCl_2 \cdot 2H_2O$ solution containing 1,300 µg NaN_3 mL^{-1} to inhibit microbial action. Then, the Teflon tubes were again shaken for 24 hours on a Reax 2 overhead shaker (Heidolph, Schwabach, Germany). This represented the first desorption step. In total, 5 desorption steps were carried out. After each desorption step the soil samples were centrifuged at 20 ± 1 °C for 25 min at 35,000 rcf and a 25 mL aliquot of the supernatant was taken and replaced by 25 mL of herbicide free 0.01 M $CaCl_2 \cdot 2H_2O$ solution containing 1,300 µg NaN_3 mL^{-1}. The sampled supernatants from the different adsorption and desorption steps were filtered through filter paper circles No. 589/1. From the filtered supernatants an aliquot of 10 mL was taken, mixed with 10 mL Ultima Gold XR and put in a scintillation counter for radioactivity measurement.

4.7.4. Glyphosate adsorption experiments using PW approach

Sterilization of the soil samples and preparation of the ^{14}C-glyphosate standard solution were carried out according to the description in **4.7.1.** A volume of 0.090 mL of the application standard was applied to 3.5 g sterilized soil in a 100 mL glass beaker in order to obtain a final glyphosate concentration of 10 µg g $soil^{-1}$. The soils were compacted to a soil density of 1.3 g cm^{-3} and soil water was adjusted to a water potential of -15 kPa. The beaker was covered with Parafilm and placed in a desiccator containing water at the bottom to prevent an evaporation of water from the sample for a period of 3 days. In preliminary experiments (**4.11**) a sorption equilibration period of 3 days was found to be optimal.

After the sorption equilibration time, the soil (30 g dry weight) was transferred in a custom-built centrifuge container and then centrifuged to extract the soil pore water. A detailed description of soil pore water extraction is given in **4.3.3**. The soil samples after centrifugation were mixed well with 15.0 g diatomaceous earth. Aliquots were taken to determine the residual radioactivity in the soil after combustion in an automatic sample-oxidizer (adsorbed amount of glyphosate) (**4.3.5**). Dissolved amount of glyphosate was determined as the amount of glyphosate in aqueous phase. In order to have a correct calculation for the dissolved and adsorbed glyphosate after adsorption experiment, the proportion of dissolvable fraction which remained in the soil after centrifugation was

calculated. This proportion was added to the dissolvable glyphosate and subtracted from the radioactivity that was determined by combustion (adsorbed residues).

4.7.5. Glyphosate desorption experiments using PW approach

The soils Apace-njiva, Skrinjar and Hohenwart were selected as 3 reference soils for desorption experiments since these soils have nearly the same soil pH, but show a relatively strong variation in their amounts of Al concentration. These 2 soil parameters have been the selection criteria since they are the factors most strongly governing sorption and desorption of glyphosate in soil.

Sterilization of the soil samples and preparation of the ^{14}C-glyphosate standard solution were carried out according to the description in **4.7.1** and **4.3.1**. Herbicide application procedure was the same as described in **4.7.4**.

After a sorption equilibration time of 24 hours, the soil was transferred to a custom-built centrifuge container, and centrifuged (**4.7.4**). Immediately after this initial adsorption step, 15 desorption steps were started: after centrifugation, the soil samples in the upper centrifuge container were de-compacted carefully with a spatula before the soil was compacted again to soil density of 1.3 g cm^{-3}. The water was re-compensated to reach a water potential of -15 kPa. The added amount of water, defined as the loss of water via evaporation during centrifugation process plus the extracted amount of water after centrifugation, contained also NaN_3 to prevent microbial degradation of glyphosate during the desorption process (195 mg NaN_3 in total amount of water at water potential of -15kPa). Finally, the cup was closed tightly with aluminum cap and placed in a desiccator. After an incubation period of 24 hours, the soil samples were extracted again via centrifugation to determine desorbed amount of ^{14}C-glyphosate in soil pore water (**4.3.3**).

4.8. Soil respiration experiments

Microbial respiration was measured in the 21 different agricultural soils that were used for the study. The experiments were run in 5 replicates for each soil sample in Respicond at the same conditions as for the biodegradation experiments (-15 kPa, 1.3 g cm^{-3}, 20 ± 1 °C). The Respicond is an instrument that can "simultaneously" measure the respiration rate of 96 soil samples. The samples are incubated in a large water bath at a constant temperature of 20 ± 1 °C. The CO_2 evolved by the sample is absorbed by a potassium hydroxide solution (0.06

M KOH), inducing the following chemical reaction: $2KOH + CO_2 \rightarrow K_2CO_3 + H_2O$. This changes the conductivity of that solution which is measured across platinum electrodes and recorded automatically by a computer at defined time intervals.

Microbial respiration was measured in soil samples (50 g dry mass) with glyphosate and without glyphosate to investigate whether there is any effect of glyphosate on soil respiration. For the glyphosate treated samples (10 µg glyphosate g^{-1} soil) a volume of 0.05 mL of the glyphosate application standard solution with a concentration of 10,000 µg μL^{-1} was applied to an oven dried, pulverized soil aliquot of 3.5 g (dry mass) in a 50 mL glass beaker. The aliquot was carefully stirred for 1 minute with a spatula. After homogenous distribution the spiked aliquot was transferred to a 500 mL Respicond incubation vessel containing the rest of equilibrated soils (46.5 g dry mass). The total soil of 50 g was homogenized again for another 2 min, compacted to a density of 1.3 g cm^{-3} and water was supplied to reach a water potential of -15 kPa. The incubation vessels were placed in the water bath of a Respicond V (Thermo Electro, Karlsruhe, Germany) for 32 days in the dark at 20 ± 1 °C. The water in the water bath was circulated by a thermostat (HAAKE DC30/DL30-Thermo Electron Corporation, Karlsruhe GmbH, Germany) and the water temperature was regulated by a through-flow cooler (DLK 10, Lauda-Königshofen, Germany). A small beaker, filled with 10 mL of 0.06 M Potassium hydroxide was placed below the incubation vessel´s cap to capture CO_2 released through soil microbial respiration from the soil samples. By trapping the evolved CO_2, the conductivity of the 0.06 M KOH solution was changed. The conductivity of each vessel was measured every hour across platinum electrodes and recorded automatically by a computer. The potassium hydroxide solution was exchanged three times per week until end of the experiment. The control treatment with no application of glyphosate was done parallel in the same manner as the glyphosate application treatment. The measurements were calculated to final unit of µg released CO_2 g^{-1} soil day^{-1}.

4.9. Establishing sterile soil conditions to prevent microbial action

For being able to quantify the sorption and desorption of the pesticide glyphosate in soil samples for both "OECD approach" and "PW approach", it was essential to avoid any microbial action like degradation processes in these soils because those microbial processes would affect the pesticide sorption and desorption dramatically. Although glyphosate sorption and desorption are rapid processes in soils (Sprankle et al., 1975b; Feng and Thompson, 1990; Tu et al., 2001), degradation and mineralization of glyphosate seems to be even faster

(Nomura and Hilton 1977; Moshier and Penner, 1978) and therefore a main goal was to identify a proper method to avoid any microbial action in soils without artificially modifying the sorption and desorption capabilities of soils. It was not possible to sterilize the soils via radiation because with the radiation sterilization, soil properties may also be changed; autoclaving of soils seems not to be a proper method, as this strange procedure will artificially change the three-dimensional structure of organic matter (Trevors, 1996) and thus most likely also the sorption behavior of glyphosate in soils as well.

Therefore, "sterile conditions" in soils by adding the microbial toxin NaN_3 was decided to achieve. From Sorensen et al. (2006) it is known that a relatively high amount of NaN_3 must be applied to soils to hamper microbial degradation of glyphosate. Therefore, this very high NaN_3-concentration of 6,500 µg g^{-1} in soils was tested, and in order to reduce this very high amount a lower NaN_3-concentration (1,000 µg g^{-1}) was also checked. This experiment was conducted in triplicates with 30 g (dry mass) of Feldkirchen soil. This soil was selected because it showed very high microbial activity in a previous study.

4.9.1. Application of NaN_3 to the soil

Moreover, to test the most efficient procedure to homogeneously mix the NaN_3 with soil, there was a trial performed with two different approaches (direct and pre-application).

A) Direct application approach: 0.1 and 0.6 mL of stock NaN_3 with a concentration of 325 mg mL^{-1} were applied directly to 30 g soil (dry mass) stored in 250 mL glass beaker to give final concentrations of 1,000 and 6,500 µg g^{-1} soil, respectively. The soil and NaN_3 were then mixed well for 2 minutes.

B) Pre-application approach: 0.1 mL NaN_3 of stock NaN_3 with a concentration of 325 mg mL^{-1} was mixed with an oven dried, pulverized soil aliquot of 3.5 g (dry mass) in a 50 mL glass beaker. The aliquot was carefully stirred for 1 minute with a spatula. After homogenous distribution the spiked aliquot was transferred to a 100 mL glass beaker containing the rest of the equilibrated soil (26.5 g dry mass). The soil was then stirred for another 2 min. The final NaN_3 concentration was 1,000 µg g^{-1} soil.

Finally, the soil samples of the two different application approaches were transferred to 500 mL brown incubation flasks, compacted to a soil density of 1.3 g cm^{-3} and soil water was

adjusted to 75 % of a water potential of -15 kPa. Subsequently, the incubation flasks were covered with Parafilm and transferred to a desiccator containing water at the bottom to avoid water evaporation from the samples. After an equilibration time of 3 days at 20 ± 1°C in the dark, ^{14}C-glyphosate was applied to the sterilized soil samples. A volume of 0.083 mL of the ^{14}C-glyphosate standard solution (3.65 µg glyphosate µL^{-1}, specific radioactivity 332.14 Bq µg^{-1}) was applied to an oven dried, pulverized soil aliquot of 3.5 g (dry mass) in a 50 mL glass beaker and carefully stirred for 1 minute with a spatula. After homogenous distribution the spiked aliquot was transferred to a 100 mL glass beaker containing the rest of equilibrated soils (26.5 g dry mass). The total soil of 30 g was homogenized again for another 2 min. The total concentration of glyphosate was 10µg g^{-1} in each set corresponding to a total radioactivity of 100,000 Bq. The sterilized and spiked soil samples were transferred again to the 500 mL brown incubation flasks, compacted to a soil density of 1.3 g cm^{-3} and soil water was adjusted to a water potential of -15 kPa. The incubation flasks were covered with rubber caps and incubated at room temperature at (20 ± 1 °C) in the dark for 7 days. During the incubation time $^{14}CO_2$ was monitored as described in **4.3.2**.

4.9.2. Sustainability of the NaN_3 sterilizing effect

The aim of this experiment was to test the sustainability of the NaN_3 sterilizing effect in soil Feldkirchen. Soil samples were incubated for different times with the sterilizing agent NaN_3 before ^{14}C-glyphosate was applied. By soil pore water extraction and HPLC analysis the possible microbial degradation of glyphosate after the different NaN_3 equilibration times was checked. The experiment was repeated for 15 replicates.

0.6 mL NaN_3 of stock NaN_3 with a concentration of 325 mg mL^{-1} was applied to 30 g (dry mass) soil via the pre-application approach (**4.9.1**) resulting in a NaN_3 concentration of 6,500 µg g^{-1} soil. The sterilized soil samples were equilibrated in a desiccator at 20 ±1 °C in the dark. After different equilibration times of 1, 2, 3, 4, and 7 days, three replicates were removed from the desiccator and ^{14}C-glyphosate was applied: a volume of 0.33 mL of the ^{14}C-glyphosate application standard with a concentration of 0.84 µg µL^{-1} and a specific radioactivity of 281.05 Bq µg^{-1} was added an oven dried, pulverized sterilized soil aliquot of 3.5 g (dry mass) in a 50 mL glass beaker and carefully stirred for 1 minute with a spatula. After homogenous distribution the spiked aliquot was transferred to a 100 mL glass beaker containing the rest of equilibrated soils (26.5 g dry mass). The total soil of 30 g was homogenized again for another 2 min. The total concentration of glyphosate was 10µg g^{-1} in

each set corresponding to a total radioactivity of 83,000 Bq. The spiked soil was placed back to 500 mL brown incubation flask, compacted to a soil density of 1.3 g cm^{-3} and soil water was adjusted to a water potential of -15 kPa. The incubation flask was covered with Parafilm and placed in a desiccator for 24 hours at 20 ±1 °C in the dark. After 24 hour incubation with glyphosate in a desiccator, the samples were centrifuged to extract the soil pore water (**4.3.3**). To quantify the radioactivity in the extracted pore water, 0.1 mL of the extracted soil pore water were mixed with 5 mL of the scintillation cocktail Ultima Gold XR and measured in a Tricarb 1900 TR scintillation counter (Packard,Dreieich, Germany). Finally, the rest amount of soil pore water was concentrated and cleaned for HPLC analysis (**4.3.4**).

4.10. Sorption equilibrium time of glyphosate in soils with OECD approach

The aim of this experiment was to identify the sorption equilibrium time of glyphosate on Lomanose soil under an excess of water (OECD approach). This soil was selected for the experiment since it has relatively high amount of clay content. Experiment was run with 28 replicates.

Before application of glyphosate the soil samples were sterilized according to **4.7.1.** A ^{14}C-glyphosate standard solution with a concentration of 0.887 µg μL^{-1} and a specific radioactivity of 11.89 Bq μg^{-1} was used. A volume of 0.074 mL of the application standard was applied to 7 g sterilized soil in a 40 mL Teflon tube in order to reach a final concentration of glyphosate in the soil of 10 µg g^{-1} soil. Furthermore, the rest of liquid phase (35 mL 0.01M $CaCl_2$ in total) was added to obtain the ratio mass between soil matrix and liquid phase of 1:5. The tubes were placed on a Reax 2 overhead shaker (Heidolph, Schwabach, Germany) under room temperature (20 ±1 °C) and shaken for different times). Four replicates of soil samples were taken after shaking for 2; 4; 8; 12; 16; 20 and 24 hours, respectively. The samples were centrifuged and processed as described in **4.7.2.**

4.11. Sorption equilibrium time of glyphosate in soils with PW approach

The aim of this experiment was to determine the sorption equilibrium time of herbicide glyphosate in 3 different clay soils: Neumarkt, Lomanose and Feldkirchen. For each soil 3 replicates were used.

Before application of glyphosate the soil samples were sterilized according to **4.7.1.** A volume of 0.40 mL ^{14}C-glyphosate application standard with a concentration of 0.70 µg μL^{-1}

and a specific radioactivity of 277.73 Bq μg^{-1} was applied to 30 g (dry mass) soil (refer **4.4.1** for glyphosate application). After incubation with glyphosate in a desiccator for 4, 7 and 9 days, respectively, 3 replicates of the soil samples were centrifuged to extract the soil pore water (**4.3.3**). To quantify the radioactivity in the extracted pore water, 0.1 mL of the extracted soil pore water were mixed with 5 mL of the scintillation cocktail Ultima Gold XR and measured in a Tricarb 1900 TR scintillation counter (Packard,Dreieich, Germany).

4.12. Testing the effect of 6,500 µg NaN_3 g^{-1} soil on the quality of soil pore water

The fact that no direct correlation was found between glyphosate mineralization and dissolved glyphosate in soil pore water (PW approach) arises the question ´´what could be the reason for that lacking correlation between mineralization and sorption behaviour of glyphosate by PW approach?´´. One reason could perhaps be the high NaN_3 concentration which has been taken from the literature (Sorensen et al., 2006) because according to Parochetti and Warren (1970) and Wolf et al. (1989) the soil pH significantly changed in soils treated with 200 µg NaN_3 g^{-1} soils. Moreover, Betterton (2000) and Burgess and Twigg (2006) showed NaN_3 forms a complex with transition metals, e.g. Al^{3+}, Fe^{3+}, particularly Fe (III). This complex is a freely soluble in aqueous phase with a dark red color. This reaction results in a decrease in the adsorption of glyphosate on Al/Fe-oxides sites because A/Fe-oxides in soils are clocked by NaN_3. Therefore, the experiment was conducted to check whether application of 6,500 µg NaN_3 g^{-1} soil brings to a change of soil pore water quality, subsequently, it effects on sorption behavior of glyphosate in soils. Five soils (Lamanose, Zepovci, Zepovci(Plitv.), Konjise and Brezje) which are supposed to be more influenced by NaN_3 than other soils were tested since they have very high amount of oxalate extractable Al^{3+} and Fe^{3+} as compared to other soils. Two treatments were established in this experiment: (1) control, having no application of NaN_3 and (2) NaN_3 application with a concentration of 6,500 µg NaN_3 g^{-1} soil. All treatments consisted of 3 replicates. Refer **4.9.1B** for the procedure how to apply NaN_3. After 3 days incubation, the soil pore water of soil samples was extracted (**4.3.3**). The pH and color of soil pore water after extracting procedure was observed and measured.

4.13. Data analysis

The SPSS statistical software (Windows 12.0 version, SPSS Inc., Illinois, USA) was applied to analyze the data. All the results were expressed as mean values of 4 replicates. Correlation between soil parameters and mineralization or correlation between glyphosate sorption and soil parameters, was assessed by a bivariate correlation analysis. A Multiple regression analysis was performed for checking soil parameters which control the sorption and mineralization of glyphosate in soil. For determining differences between treatments in some experiments T-test, one way Anova and Tukey HSD test analyses were applied and the differences obtained at a level of $P < 0.05$ were considered significant.

5. Results

5.1. Degradation of glyphosate in agricultural soils

5.1.1. Mineralization of glyphosate

After 32 days of incubation a big variance of cumulative mineralization can be observed in Figure 5.1a and Figure 5.1b. Between 7.6 to 68.7 % of the applied ^{14}C-glyphosate was mineralized to $^{14}CO_2$ in the 21 different soil types (Table 5.1). The lowest mineralization of ^{14}C-glyphosate was identified in Brezje soil while the highest mineralization of ^{14}C-glyphosate was obtained in Feldkirchen and Apace-njiva soils. Low mineralization of glyphosate was also observed in Zepovci, Zepovci(Plitv.) and Lamanose soils. In these 3 soils less than 30 % of the initial glyphosate was mineralized after 32 days. In contrast, other soils had a higher mineralization activity and $^{14}CO_2$ production after 32 days reached 31.2-68.7 % of the initial glyphosate. High variability of glyphosate degradation in laboratory experiments was also reported in the studies of Smith and Aubin (1993); Cheah et al. (1998); Gimsing et al.(2004a); Klier (2007). They showed that Dt50 lab values varied between 4 to 180 d (mean 49 d) at 20 ±1 °C.

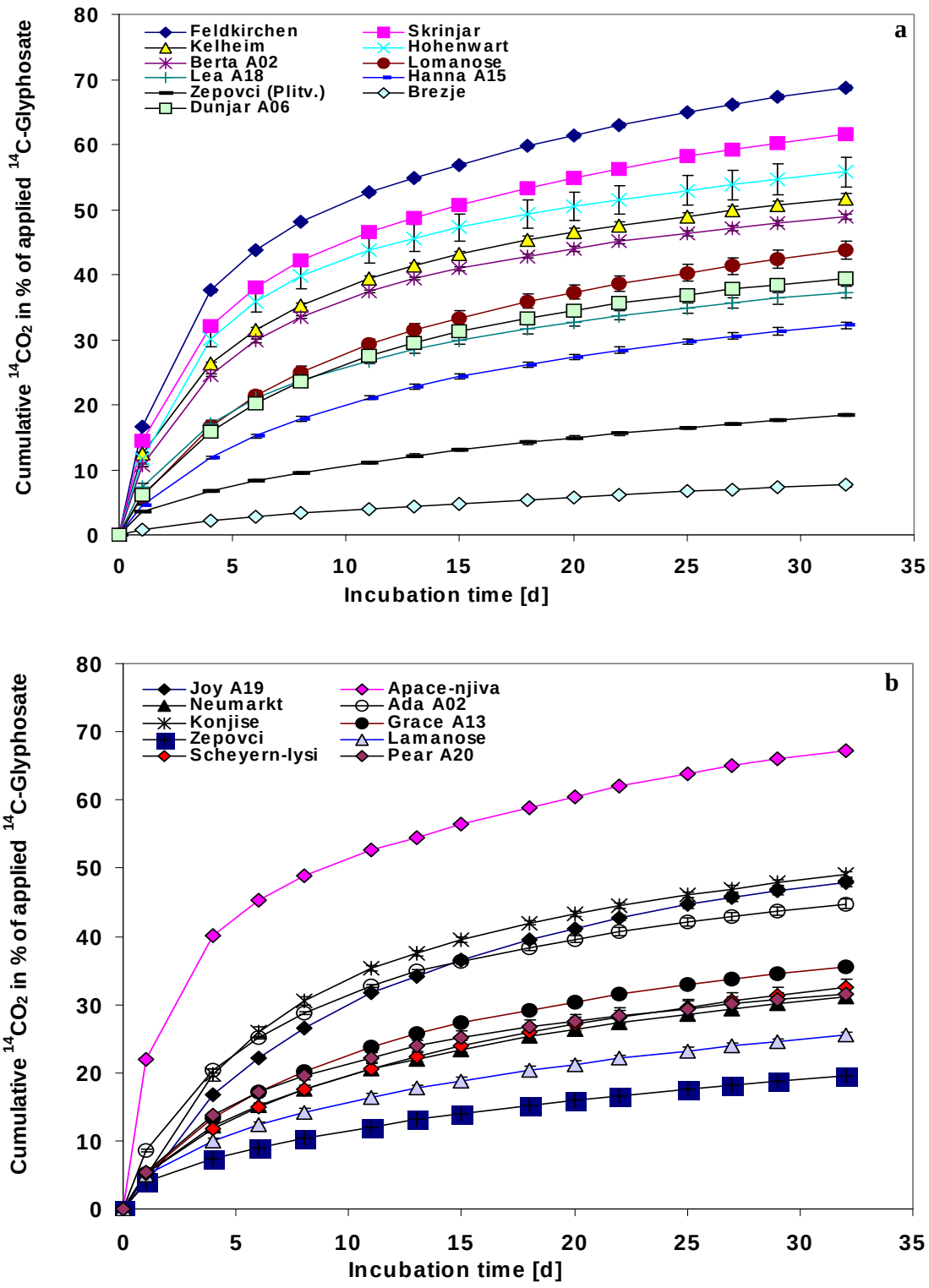


Figure 5.1. Development of cumulative mineralization of ^{14}C-glyphosate in 21 agricultural soils in course of 32 day incubation (bars indicate standard deviation)

Figures 5.2a and 5.2b show results of the daily mineralization rates of glyphosate. In general, in most of the soils daily mineralization of glyphosate was initially rapid and the highest mineralization rate is reached shortly after application, but reduced with time, except for the cases of Joy A19 and Konjise soils. In these 2 soils, the daily mineralization rate was stable during the first 4 days, afterwards it considerably dropped (Figure 5.2b). The daily mineralization rate was really low in Brezje soil (<1 % of the applied ^{14}C per day). The daily mineralization rate in most soils reduced lower than 1 % of applied ^{14}C per day between day 10 and day 15 while the daily mineralization rate in Lamanose, Zepovci and Zepovci (Plitv.) dropped lower than 1 % of applied ^{14}C per day between day 5 and day 10.

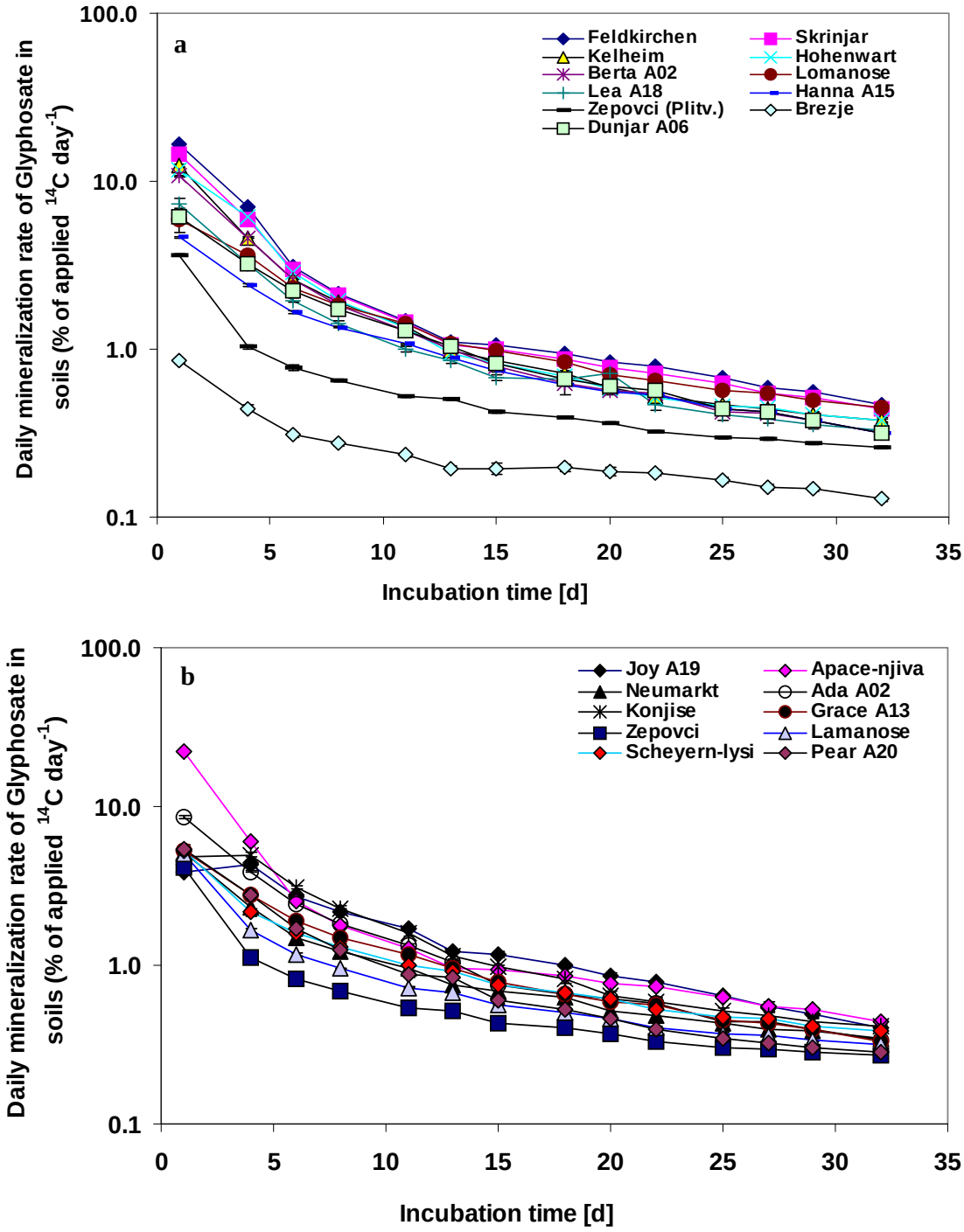


Figure 5.2. Development of daily mineralization rate of glyphosate in 21 agricultural soils in course of 32 day incubation (bars indicate standard deviation)

5.1.2. Calculation of correlations between cumulative mineralization of glyphosate and soil properties

Since one of the main objectives of this study was to identify the soil factors which govern glyphosate mineralization, several univariate correlations and a multivariate correlation between cumulative mineralization and soil parameters were calculated. The results for the univariate correlations are presented in Figure 5.3. The mineralization of glyphosate is significantly correlated to exchangeable H^+ ($p = 0.000$), soil pH ($p = 0.000$), oxalate extractable Al^{3+} (Al^{3+}_{Ox}) ($p = 0.010$), and CFU_{end} ($p = 0.003$), respectively. A negative correlation between mineralization of glyphosate with exchangeable H^+, and Al^{3+}_{Ox}, was observed whilst a positive correlation was found between mineralization with soil pH, and the cell counts at the end of the experiments (CFU_{end}). Taking all the results into account allows identifying that the mineralization of glyphosate depends very much on exchangeable H^+, soil pH, Al^{3+}_{Ox} amounts and CFU_{end}.

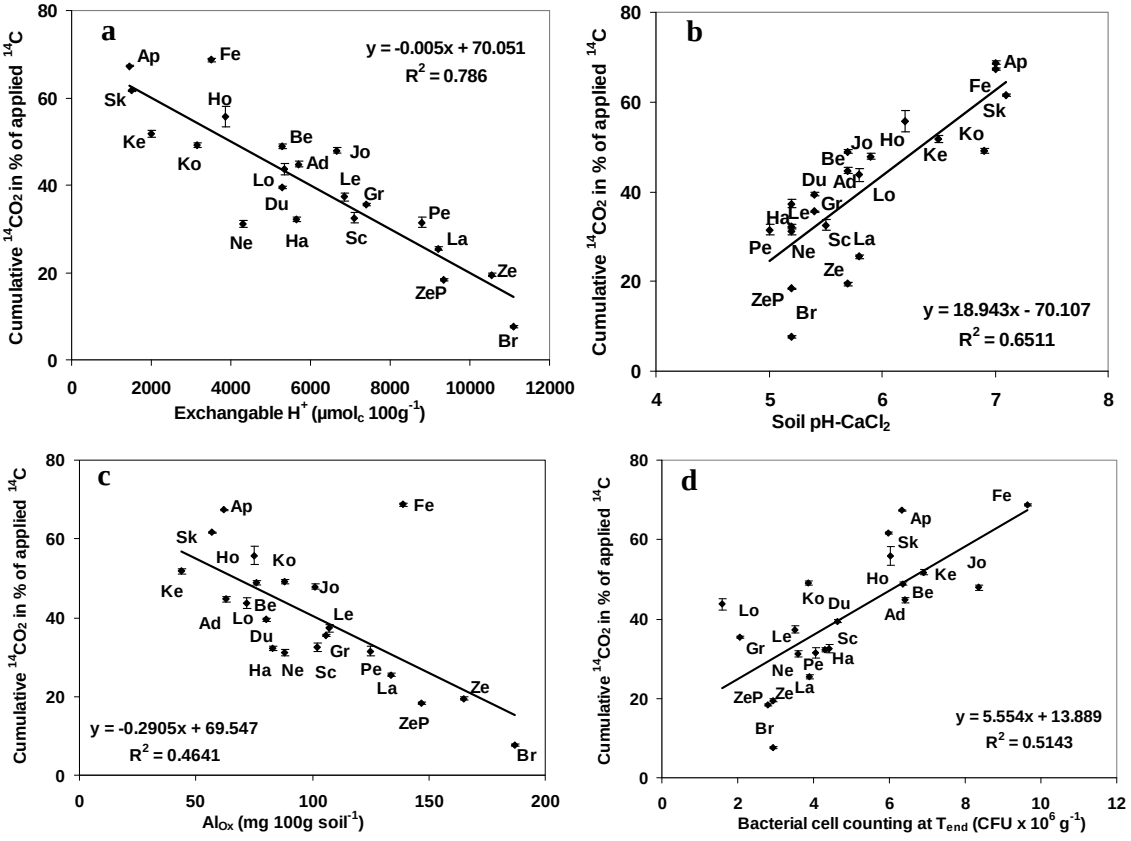


Figure 5.3. Correlations of cumulative glyphosate mineralization in course of 32 day incubation in different soils with exchangeable H^+ (a), soil pH (b), oxalate extractable Al^{3+} (c) and bacterial cell counts at the end of the experiment (d) (bars indicate standard deviation)

In order to investigate the interacting functions of the different soil parameters on cumulative glyphosate mineralization, a multiple regression analysis was used. The input parameters were dissolved glyphosate-PW, dissolved glyphosate-OECD, exchangeable [H^+], silt, clay, soil organic matter, C, N, C/N, P_2O_5, Cu^{2+}, oxalate extractable Al^{3+}, oxalate extractable Fe^{3+}, K_2O, $CFU_{beginning}$ and CFU_{end}, Ca^{2+}, Mg^{2+}, K^+, Na^+, CEC, and pH. The mineralized amount of glyphosate in the experiments was best described by the model A (n = 21):

$$Gly_{cum.min}\ [\%] = -0.005 \times [H^+_{Exc.}] + 1.025 \times [Ca^{2+}] + 0.332 \times [K_2O] + 56.338$$

(Adjusted $R^2 = 0.90$, $p = 0.25 \times 10^{-9}$)

$[H^+_{Exc.}]$ is exchangeable H^+ in soils (μmol_c 100 g^{-1} soil),

$[Ca^{2+}]$ is exchangeable Ca^{2+} in soils ($mmol_c$ 100 g^{-1} soil),

$[K_2O]$ is plant available potassium in soils (mg 100 g^{-1} soil)

The result of multiple regression analysis reveals exchangeable [H^+], [Ca^{2+}] and [K_2O] as key parameters governing collectively glyphosate mineralization in the 21 tested soils.

5.1.3. NaOH extractable residues and correlations with soil properties

The NaOH extractable residues of glyphosate in all 21 soils are shown in Table 5.1. The NaOH extractable fraction is interpreted as the amount of glyphosate that is adsorbed to iron and aluminum oxides (Gimsing et al., 2004a). As can be seen, the NaOH extractable fraction in all soils was relatively high and very various. Approximately between 23 and 91 % of initial glyphosate after 32 days incubation was extracted with NaOH 0.1M. Soils with higher mineralization had lower NaOH extractable fraction. The highest NaOH extractable residues (91 %) were achieved in Brezje soil which had very low mineralization of glyphosate after 32 days. In contrast, only 23.3 % of initial glyphosate was extracted by NaOH in the case of Feldkirchen soil which showed very high glyphosate mineralization.

5.1.3.1. Calculation of correlations between NaOH extractable residues and soil properties

The results from univariate regression analyses are shown in Fig. 5.4, There were significant correlations between NaOH extractable residues and several soil parameters [exchangeable H^+ ($p = 0.000$), soil pH ($p = 0.0000$), oxalate extractable Al^{3+} ($p = 0.0004$),

bacterial cell counts at the end of the experiment ($p = 0.0008$), and glyphosate residues from extractable pool ($p = 0.0000$)].

There was a positive correlation between NaOH extractable glyphosate and exchangeable H^+, oxalate extractable Al^{3+} and ^{14}C-glyphosate in NaOH extractable residues, while a negative correlation of NaOH extractable glyphosate with soil pH and CFU_{end} was evident. According to univariate regression analysis exchangeable H^+, soil pH, oxalate extractable Al^{3+} and CFU_{end} are important factors governing the sorption and mineralization of glyphosate.

In order to investigate the interacting functions of the different soil parameters on NaOH extractable residues, a multiple regression analysis was used. The input parameters were exchangeable [H^+], silt, clay, soil organic matter, C, N, C/N, P_2O_5, Cu^{2+}, oxalate extractable Al^{3+}, oxalate extractable Fe^{3+}, K_2O, $CFU_{beginning}$ and CFU_{end}, Ca^{2+}, Mg^{2+}, K^+, Na^+, CEC, H^+pH and pH. The NaOH extractable residues were best described by the model B ($n = 21$):

$$\text{NaOH extractable residues [\%]} = 0.0063 \times [H^+_{Exc.}] - 1.02 \times [\text{CEC}] + 32.06$$

$$(\text{Adjusted } R^2 = 0.91,\ p = 2.70 \times 10^{-10})$$

$[H^+_{Exc.}]$ is exchangeable H^+ in soils ($mmol_c\ 100\ g^{-1}$ soil),

[CEC] is cation exchange capacity in soils ($mmol_c\ 100\ g^{-1}$ soil)

The result of multiple regression analysis reveals that $[H^+_{Exc.}]$ and CEC are the most important factors contributing collectively to the formation of NaOH extractable residues in the 21 investigated soils.

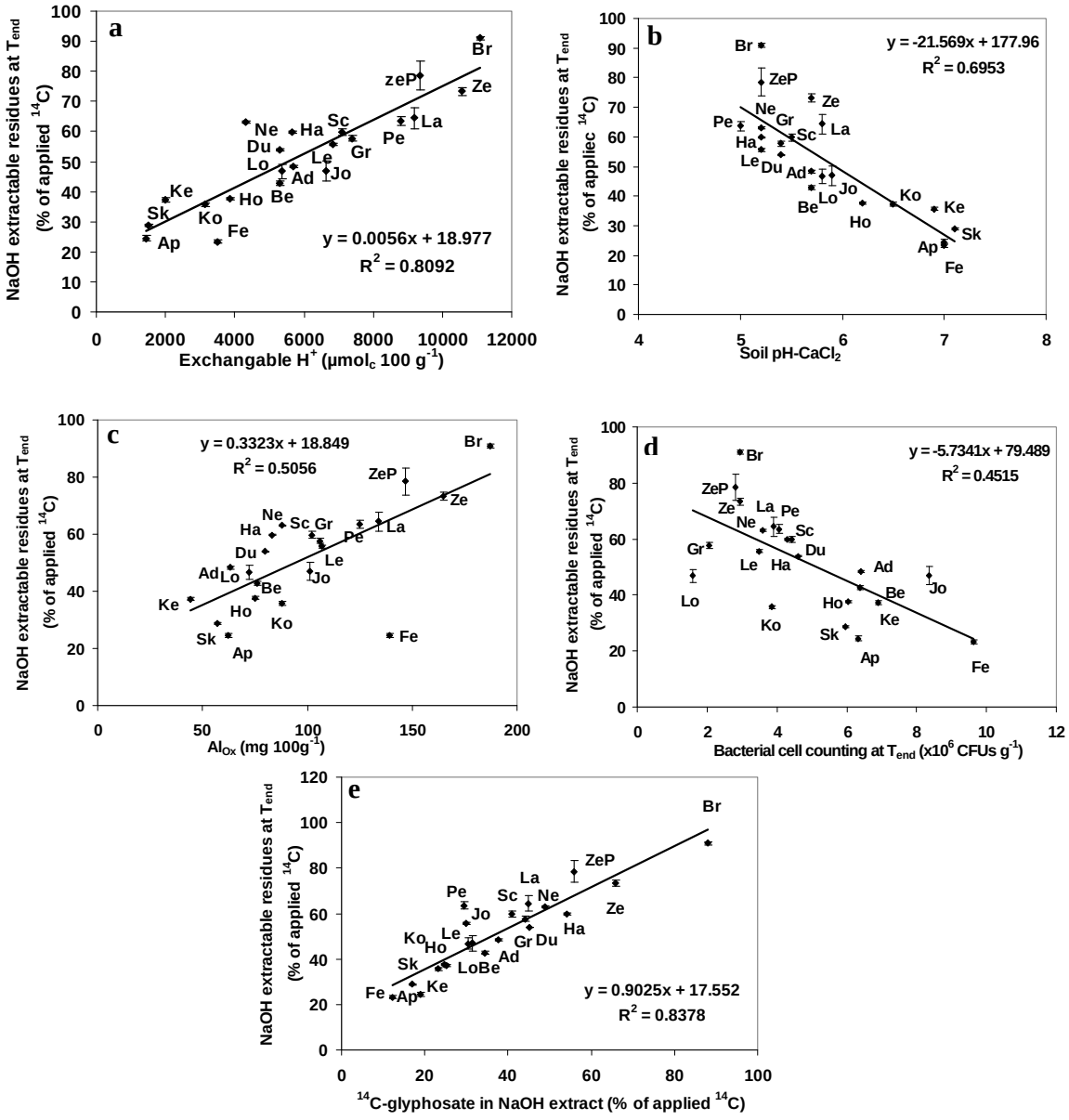


Figure 5.4. Correlations of NaOH extractable glyphosate at the end of the biodegradation experiments with exchangeable H^+ (a), soil pH (b), oxalate extractable Al^{3+} (c), bacterial cell counts at the end of the biodegradation experiments (d) and ^{14}C-glyphosate in NaOH extractable residues (e) (bars indicate standard deviation)

A strong and negative correlation (p = 0.0000) between cumulative mineralization of glyphosate within 32 days and NaOH extractable residues at the end of the experiments was found (Figure 5.5).

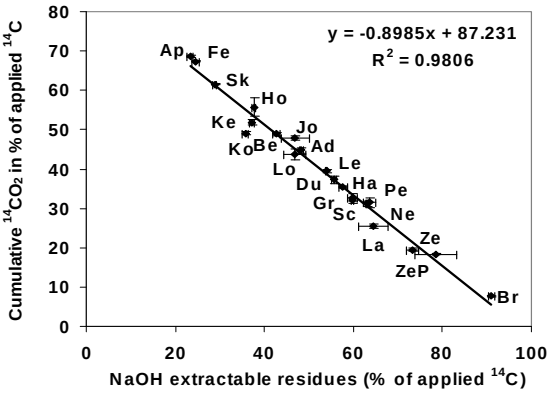


Figure 5.5. Correlation of NaOH extractable glyphosate at the end of the biodegradation experiments with cumulative mineralization of glyphosate in course of 32 day incubation (bars indicate standard deviation)

5.1.3.2. Quality of NaOH extractable residues at the end of the experiments

Table 5.2 shows additionally the quality of NaOH extractable fraction after 32 days incubation period. The results reveal that glyphosate is a major component in NaOH extracts, relatively high, between 12.2 to 88 % of initial glyphosate. In Brezje soil, which showed the lowest mineralization, the component of glyphosate in NaOH extract was 88 % while in Feldkirchen soil which showed the highest mineralized amount had 12.2% of glyphosate in NaOH extract. The major metabolite could not be identified and is named ”Unknown” in Table 5.2. This metabolite varied among soils between 3.0 to 34.1 %. There was very little AMPA in the NaOH extract at the end of the experiment which varied between 0 to 11.3 % of the initial glyphosate. AMPA concentrations below the detection limit were found in of the soils Berta A02, Brezje, Dunja A06, Hanna A15, Lea A18, Pear A20 and Zepovci.

Table 5.1. Cum. mineralization, NaOH extractable residues, non extractable residues, residues in pore water and ^{14}C mass balance of glyphosate after 32 days of incubation of the 21 soils

Soil	Cum. min (%)[a)]	NaOH extractable residues (%)[a)]	Non extractable residues (%)[a)]	Total recovery (%)[a)]
Ada A02	44.7 (±0.7)	48.3 (±0.4)	4.8 (±0.1)	97.8 (±0.9)
Apace-njiva	67.3 (±0.1)	24.5 (±0.8)	9.6 (±0.2)	101.4 (±0.8)
Berta A02	48.9 (±0.4)	42.7 (±0.7)	6.3 (±0.5)	97.9 (±0.5)
Brezje	7.6 (±0.2)	91.0 (±0.7)	2.5 (±0.6)	101.1 (±0.3)
Dunja A06	39.5 (±0.3)	53.9 (±0.2)	3.9 (±0.1)	97.3 (±0.4)
Feldkirchen	68.7 (±0.4)	23.3 (±0.6)	9.0 (±0.2)	101.0 (±0.4)
Grace A13	35.5 (±0.3)	57.7 (±1.0)	3.7 (±0.2)	96.9 (±0.8)
Hanna A15	32.2 (±0.5)	59.8 (±0.3)	4.1 (±0.2)	96.1 (±0.6)
Hohenwart	55.8 (±2.3)	37.7 (±0.5)	6.3 (±0.3)	99.8 (±2.9)
Joy A19	47.9 (±0.6)	46.9 (±3.2)	8.2 (±0.7)	103.0 (±5.4)
Kelheim	51.8 (±0.7)	37.3 (±0.6)	6.2 (±0.4)	95.3 (±0.9)
Konjise	49.1 (±0.5)	35.7 (±0.6)	9.5 (±0.6)	94.3 (±1.4)
Lamanose	25.5 (±0.5)	64.4 (±3.3)	6.7 (±0.5)	96.6 (±3.0)
Lea A18	37.3 (±0.9)	55.7 (±0.5)	5.4 (±0.1)	98.4 (±0.9)
Lomanose	43.7 (±1.4)	46.8 (±2.5)	6.4 (±0.4)	96.9 (±2.5)
Neumarkt	31.2 (±0.8)	63.0 (±0.3)	3.1 (±0.2)	97.3 (±1.1)
Pear A20	31.5 (±1.2)	63.6 (±1.5)	3.7 (±0.2)	98.8 (±2.8)
Scheyern-lysi	32.5 (±1.2)	59.8 (±1.2)	5.0 (±0.1)	97.3 (±2.3)
Skrinjar	61.6 (±0.2)	28.8 (±0.4)	11.4 (±0.2)	101.8 (±0.5)
Zepovci	19.5 (±0.4)	73.3 (±1.4)	4.1 (±0.2)	96.9 (±1.6)
Zepovci(Plitv.)	18.4 (±0.2)	78.5 (±4.7)	2.7 (±0.3)	99.6 (±5.8)

a) % of applied ^{14}C-glyphosate after 32 days; the mean value is presented and the values in parentheses are standard deviation

Table 5.2. Quality of NaOH extractable residues in the 21 soils after 32 days of incubation (% of applied ^{14}C-glyphosate)

Soil	Quality of NaOH extractable pesticide residues[a)]		
	Glyphosate (%)	AMPA[b)] (%)	Unknown (%)
Ada A02	37.7	2.3	8.3
Apace-njiva	18.9	2.2	3.4
Berta A02	34.4	< LOD[c)]	8.3
Brezje	88.0	< LOD[c)]	3
Dunja A06	45.2	< LOD[c)]	8.7
Feldkirchen	12.2	7.6	3.6
Grace A13	44.1	2.8	10.8
Hanna A15	54.0	< LOD[c)]	5.8
Hohenwart	24.8	5.7	7.3
Joy A19	31.3	7.1	8.5
Kelheim	25.1	4.1	8.1
Konjise	23.3	4.5	7.9
Lamanose	45.0	8.3	11.1
Lea A18	30.0	< LOD[c)]	25.7
Lomanose	30.4	8.0	8.4
Neumarkt	48.8	2.5	11.7
Pear A20	29.5	< LOD[c)]	34.1
Scheyern-lysi	40.9	5.5	13.4
Skrinjar	16.9	4.8	7.1
Zepovci	65.8	< LOD[c)].	7.5
Zepovci(Plitv.)	55.9	11.3	11.3

[a)] The quality of NaOH extractable pool was detected by HPLC

[b)] Aminomethylphosphonic acid

[c)] LOD = limit of detection

5.1.4. Non extractable residues and correlations with soil properties

Non extractable glyphosate residues are considered as the bound residues in soils after NaOH extraction. The amount of non extractable residues was relatively low (Table 5.1). It varied between 2.5 % and 11.4 % of the initial glyphosate. In Brezje soil, the non extractable

residues were 2.5 % of the initial glyphosate while those of Skrinjar were 11.4 %, the highest amount that was found in the different soils.

According to univariate correlation analysis (Figure 5.6) the non extractable residues at the end of the experiment were negatively correlated with exchangeable H^+ ($p = 0.0005$), but positively correlated with soil pH ($p = 0.0000$). It means that the non-extractable residues were lower in soils with low pH and high exchangeable H^+.

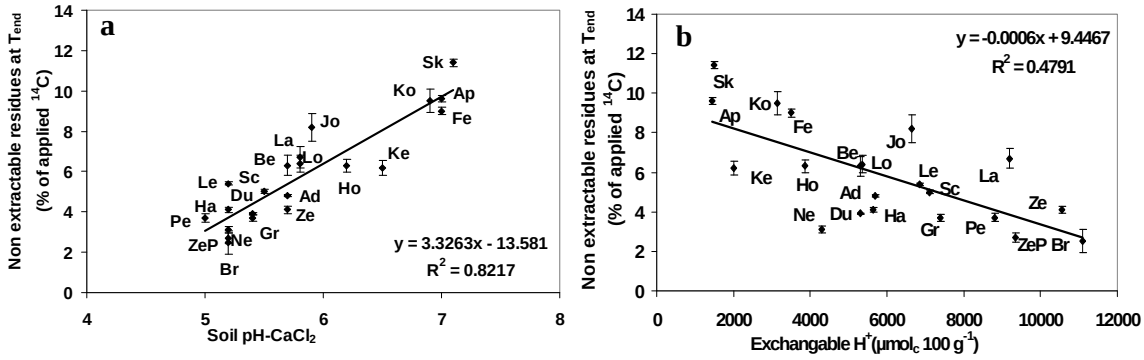


Figure 5.6. Correlations of non extractable residues at the end of the biodegradation experiments with soil pH (a) and exchangeable [H^+] (b) (bars indicate standard deviation)

In order to investigate the interacting functions of the different soil parameters on non extractable residues, a multiple regression analysis was used. The input parameters were exchangeable [H^+], silt, clay, soil organic matter, C, N, C/N, P_2O_5, Cu^{2+}, oxalate extractable Al^{3+}, oxalate extractable Fe^{3+}, K_2O, $CFU_{beginning}$ and CFU_{end}, Ca^{2+}, Mg^{2+}, K^+, Na^+, CEC, and pH. The non extractable residue was best described by the model C (n = 21):

$$\text{Non extractable residues [\%]} = 2.92 \times \text{pH} + 0.13 \times [\text{Water content at -15 kPa}] - 0.02 \times [Al_{Ox}] - 12.30$$

$$(\text{Adjusted } R^2 = 0.89,\ p = 6.62 \times 10^{-9})$$

$[Al_{Ox}]$ is oxalate extractable Al^{3+} (mg 100 g^{-1} soil)

The result of multiple regressions reveals that soil pH, water content at -15 kPa and oxalate extractable Al^{3+} are the most important factors contributing collectively to the non extractable glyphosate in the 21 investigated soils.

Figure 5.7 presents the correlation (p = 0.0000) between cumulative mineralization of glyphosate and non extractable residues. A significant and positive correlation between cumulative mineralization of glyphosate within 32 days and non extractable residues was found.

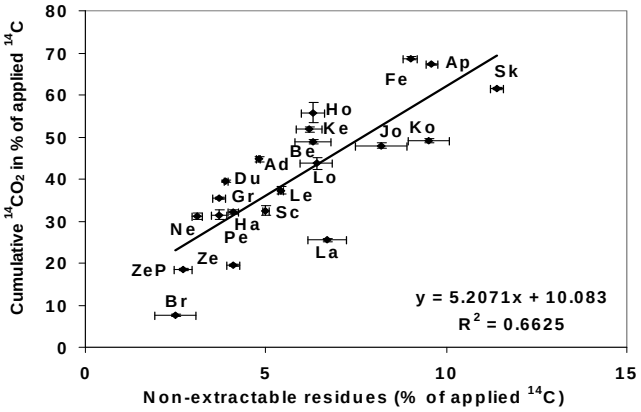


Figure 5.7. Correlation of non extractable glyphosate at the end of the biodegradation experiments with cumulative mineralization of glyphosate in the course of 32 day incubation (bars indicate standard deviation)

5.1.5. ^{14}C-glyphosate residues in soil pore solution

At the end of the experiments glyphosate residues in soil pore solution were determined using the PW approach (**4.3.3**). Glyphosate in pore solution should represent the bioavailable fraction of glyphosate. As presented in Table 5.1, after 32 days of incubation glyphosate residues in soil pore solution were very low, with a variance between 0.02 and 0.37 % of the applied glyphosate. There was no reasonable correlation between glyphosate in pore solution and mineralization rate at the end of the experiment (Figure 5.8).

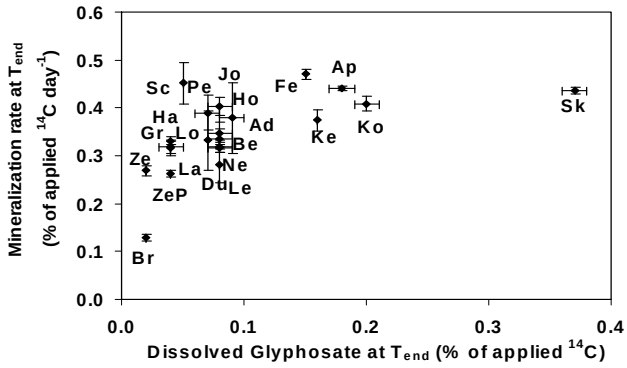


Figure 5.8. Correlation of dissolved glyphosate (PW approach) in soil pore solution and mineralization rate at the end of the biodegradation experiments (bars indicate standard deviation)

5.1.6. ^{14}C-mass balance after the experiment

Mass balances of ^{14}C-glyphosate are presented in Table 5.1. In all soils, the ^{14}C mass balances were quite good: over 94 % of the totally applied ^{14}C-glyphosate was recovered at the end of the biodegradation experiments.

5.1.7. Bacterial cell counts at the beginning and at the end of the experiments

Bacterial cell numbers counted for all 21 soils at the beginning and at the end of the experiments are presented in Figure 5.9. The development of bacterial growth during the biodegradation experiments was quite different in the various soils. At the end of the biodegradation experiments bacterial cell numbers increased in soils: Ada A02, Apace-njiva, Berta A02, Brezje, Dunjar A06, Konjise, Skrinjar and Zepovci (Plitv.), whereas a strong decrease in bacterial cell numbers was found in soils: Grace A13, Hohenwart, Lomanose, Neumarkt, Pear A20 and Scheyern-lysi. A slight decrease or no change in bacterial cell numbers was observed in soils: Feldkirchen, Hanna A16, Joy A19, Kelheim, Lamanose, Lea A18 and Zepovci.

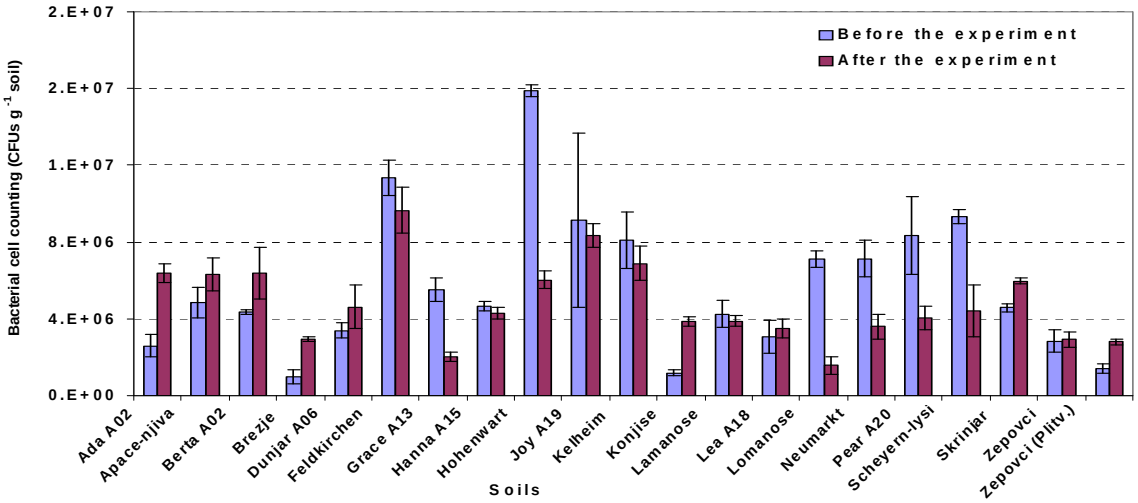


Figure 5.9. Bacterial cell counts at the beginning of the experiment (no glyphosate application) and at the end of the biodegradation experiments (with glyphosate application) (bars indicate standard deviation)

5.2. Adsorption of glyphosate in agricultural soils

For being able to determine the sorption of glyphosate in different soils with 2 different approaches (PW and OECD), some preliminary experiments were conducted to clarify for some fundamental questions regarding: (1) the best concentration of NaN_3 for inhibiting microbial action, (2) the efficient duration of the best NaN_3 concentration in soils and (3) the sorption equilibrium time of glyphosate.

5.2.1. Establishing sterile soil conditions to prevent microbial action

In the PW experiment the effect of NaN_3 to inhibit the microbial degradation of glyphosate in soils was studied. Two different concentrations of NaN_3 [(1) 1,000 and (2) 6,500 µg NaN_3 g^{-1} soil] and 2 application approaches for 1,000 µg NaN_3 g^{-1} soil [(1) direct application of NaN_3 to soils and (2) pre-application to an aliquot of 3.5 g soil (dry mass)] were tested. After application of NaN_3 to the soils, the mineralization capacity of the treated soils was monitored as a measure for the sterilization effect. The final results are shown in Figure 5.10. In the treatments with 1,000 µg g^{-1} NaN_3 the sterilizing capability of NaN_3 was apparently not efficient. In these treatments a cumulative mineralization over 7 days of 4.0 % and 9.5 % was measured, depending on the NaN_3 application method: when NaN_3 was applied to a soil aliquot which then was mixed with the whole amount of soil, the sterilizing effect of NaN_3 was more pronounced than in the case where NaN_3 was directly applied to the total amount of soil. The conclusion that can be drawn from this result is that applying NaN_3 on a 3.5 g dry soil aliquot is a better approach for introducing NaN_3 homogenously into soils. In contrast, the cumulative glyphosate mineralization was very low (below 0.5 % of applied ^{14}C-glyphosate) in the treatment with 6,500 µg NaN_3 g^{-1} soil. These results allow concluding that at a concentration of 6,500 µg g^{-1} soil, NaN_3 sufficiently inhibits the microbial activity in soils. Therefore, (1) 6,500 µg NaN_3 g^{-1} soil as it is already suggested in literature by Sorensen et al. (2006) and (2) 3.5 g dry soil aliquot for applying NaN_3 have been used in the sorption experiments (PW and OECD) during this study.

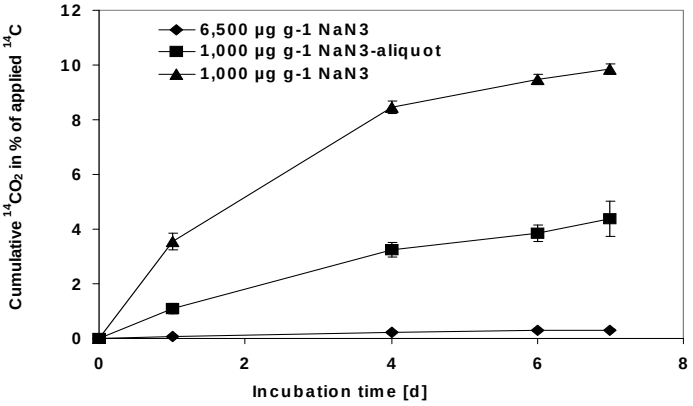


Figure 5.10. Development of CO_2 emission from mineralization of ^{14}C-glyphosate in soil Feldkirchen by different concentrations of NaN_3 and different application procedures (bars indicate standard deviation).

5.2.2. The sustainability of the sterilizing effect of 6,500 µg g^{-1} NaN_3 in soil

It is very essential to know the duration of this NaN_3 effect. Therefore, the following analysis was conducted: the extracted pore water (PW approach) was concentrated and finally the concentrated extract was cleaned up before injecting to HPLC. The main objective of the HPLC analysis was to qualify the pore water extract during different experimental times. The final results from HPLC chromatograms indicate that after 1, 2, 3, 4 and even 7 days of incubation no degradation products of glyphosate (e.g. aminomethylphosphonic acid [AMPA]) in soil pore water samples could be detected. Those results indicate that the effect of 6,500 µg NaN_3 g^{-1} soil on soil microbial activity was lasting at least up to 7 days after it was introduced into soils. Therefore, a time period of 3 days to pre-incubate 6,500 µg NaN_3 g^{-1} soil in soils was selected (before ^{14}C-glyphosate was applied) when working with PW approach because after 1, 2, 3, 4 and 7 days no degradation products were found and the reason why 3 days for the NaN3 pre-incubation period was chosen was to ensure that the sorption and desorption of glyphosate in soils are balanced to reach the equilibrium between the concentration of glyphosate in soil and soil solution for all 21 soils.

5.2.3. Sorption equilibrium time of glyphosate in soil with the OECD approach

The experiment was conducted according to the OECD guideline 106. To avoid microbial degradation of glyphosate, 6,500 µg NaN_3 g^{-1} soil were applied (according to **5.2.1** and **5.2.2**) As can be seen in Figure 5.11, glyphosate was rapidly adsorbed in soil Lomanose, only 2.39 % of applied glyphosate was dissolved in water phase after 2 hours shaking. The sorption equilibrium of glyphosate in soil Lomanose was reached in 4 to 16 hours when approximately 2.0 % of applied glyphosate remained in water phase. There was no significant difference regarding the sorption of glyphosate among incubation time spans (via the post-hoc tests [Tukey Honestly Significant Difference (HSD)]. These results are also in accordance with many earlier studies. Jesen et al. (2009) found that in 3 Chilean soils, the adsorption of glyphosate occurred rapidly and the equilibrium was reached after 2 hours while Autito et al. (2004) showed that the equilibrium between the concentration of glyphosate between soil and supernatant was reached within 16 hours in 2 Finnish soils. Thus, to ensure that the adsorption equilibrium will be reached for all 21 soils in the sorption experiments with OECD approach 16 hours was selected as reference shaking time.

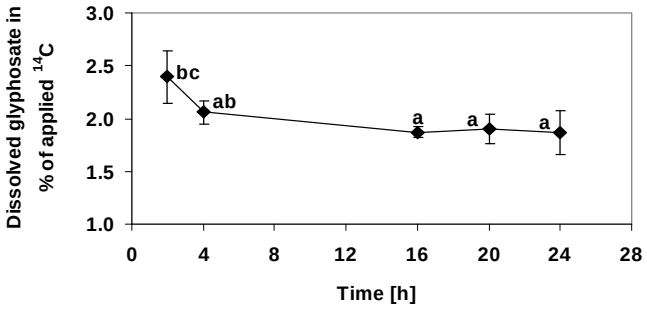


Figure 5.11. Adsorption of glyphosate in soil Lomanose in course of 24 hour incubation (OECD approach), the letters express the statistical differences between sampling time. Values with the same letter assigned are not significantly different (α = 0.05) according to one way Anova and Tukey HSD test.

5.2.4. Sorption equilibrium time of glyphosate in soils with the PW approach

The experiment was conducted according to **5.2.1** and **5.2.2**. In order to avoid the microbial degradation of glyphosate during the experiment, 6,500 µg NaN_3 g^{-1} soil were applied. The results are shown in Figure 5.12. The *in situ* sorption of glyphosate herbicide in 3 different soils was quite high. The results show that after 4 days incubation between 1.9 to 2.4 % of applied glyphosate was dissolved in PW. When comparing the dissolved amount of glyphosate in the same soil between sampling times, the post-hoc tests (Tukey HSD) revealed that no statistically significant differences between 4 days and 7 days equilibration time in any soil was found. Therefore, a time span of 4 days was selected for achieving the pesticide sorption equilibrium in soil.

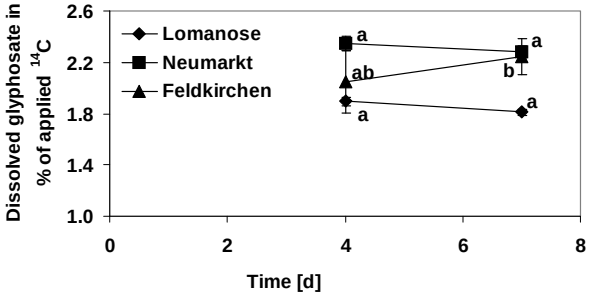


Figure 5.12. Adsorption of glyphosate in soils Feldkirchen, Lomanose and Neumarkt in course of 7 day incubation (PW approach), the letters express the statistical differences between sampling times within a soil. Values with the same letter assigned are not significantly different (α = 0.05) according to one way Anova and Tukey HSD test.

5.2.5. Dissolution and adsorption behavior of glyphosate in soils

In this study, 2 approaches were applied to determine the dissolution and adsorption of glyphosate in soils: (1) OECD approach and (2) the PW approach. The aim of this study was to find out which soil parameters govern the sorption behavior of glyphosate.

5.2.5.1. $OECD_{ad}$ approach

The adsorption experiment was conducted under sterilized conditions to inhibit microbial action by applying 6,500 µg NaN_3 g^{-1} (according to **5.2.1** and **5.2.2)**. Figure 5.13 contains the dissolution of glyphosate in 21 soils. Dissolved amounts of glyphosate in water were relatively low in all soils, between 3.5 % and 23.0 % of the initial glyphosate. A relatively high glyphosate dissolution was found in soils Kelheim and Skrinjar, approximately 23 % of the total applied glyphosate while the dissolved amount of glyphosate was evidentially very low in soil Brezje (3.5 % of the initial glyphosate).

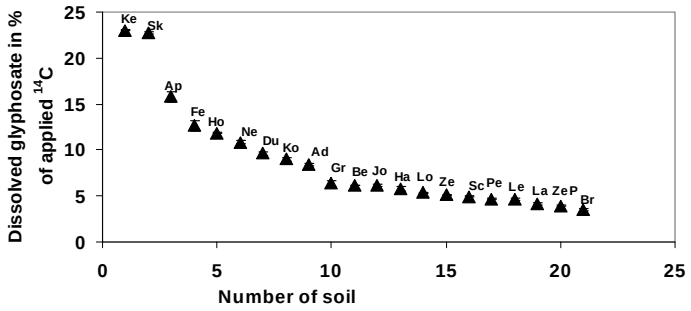


Figure 5.13. The relative glyphosate dissolution ranking of the 21 investigated soils with OECD approach

In general, glyphosate adsorption in soil is quite high. The highest adsorption of glyphosate was obtained in soil Brezje, almost 100 % of totally applied glyphosate, whereas approximately 77 % of initial glyphosate was absorbed in soils Kelheim and Skrinjar. The adsorption of other soils was more than 80 % of the initial glyphosate. In all soils, mass balance of ^{14}C-glyphosate was quite good, over 98 % of totally applied ^{14}C-glyphosate for all soils was recovered after the sorption experiments (Table 5.3).

Table 5.3. Dissolution, adsorption and recovery of glyphosate in the 21 soils with OECD approach

Soil	OECD approach		
	Dissolved amount (%)[a]	**Adsorbed amount (%)[a]**	**Total recovery (%)[a]**
Ada A02	8.4 (±0.1)	92.4 (±1.2)	100.8 (±1.2)
Apace-njiva	15.8 (±0.0)	84.6 (±1.1)	100.4 (±1.1)
Berta A02	6.2 (±0.2)	92.3 (±2.1)	98.5 (±2.1)
Brezje	3.5 (±0.1)	99.9 (±1.0)	103.4 (±0.9)
Dunja A06	9.6 (±0.1)	91.6 (±1.5)	101.2 (±1.5)
Feldkirchen	12.6 (±0.6)	89.8 (±2.6)	102.5 (±2.6)
Grace A13	6.4 (±0.1)	97.8 (±0.6)	104.2 (±0.6)
Hanna A15	5.8 (±0.1)	94.8 (±1.3)	100.6 (±1.3)
Hohenwart	11.9 (±0.6)	91.3 (±0.9)	103.2 (±0.9)
Joy A19	6.1 (±0.1)	94.7 (±0.5)	100.8 (±0.5)
Kelheim	23.0 (±0.3)	75.6 (±4.1)	98.6 (±4.1)
Konjise	9.0 (±0.3)	94.4 (±2.4)	103.4 (±2.4)
Lamanose	4.2 (±0.1)	100.3 (±0.8)	104.5 (±0.8)
Lea A18	4.6 (±0.0)	95.3 (±1.1)	99.9 (±1.1)
Lomanose	5.4 (±0.1)	98.8 (±0.3)	104.2 (±0.3)
Neumarkt	10.8 (±0.1)	90.9 (±0.5)	101.7 (±0.5)
Pear A20	4.7 (±0.1)	95.5 (±1.8)	100.2 (±1.8)
Scheyern-lysi	4.9 (±0.1)	99.6 (±0.0)	104.5 (±0.0)
Skrinjar	22.7 (±0.2)	79.0 (±1.3)	101.7 (±1.3)
Zepovci	5.1 (±0.2)	97.3 (±1.1)	102.4 (±1.1)
Zepovci(Plitv.)	3.9 (±0.0)	98.6 (±1.2)	102.6 (±1.2)

a) % of applied ^{14}C-glyphosate; the mean value is presented and the values in parentheses are standard deviation

5.2.5.2. PW approach

The experiment was conducted according to **5.2.1** and **5.2.2.** 6,500 μg NaN_3 g^{-1} soil were applied to avoid microbial degradation of glyphosate in soils. Figure 5.14 presents the dissolution of glyphosate in pore water of 21 soils. In general, dissolved amounts of glyphosate in soil pore water were very low in all soils, between 0.4 to 3.9 % of the initial glyphosate. There was a big difference between both methods used. Compared with the OECD approach, the dissolved glyphosate in soil solution was much lower with PW approach. The highest glyphosate dissolution was found in soils Kelheim, Zepovci, Zepovci(Plit.), and Lamanose, between 3 and 4 % of the totally applied glyphosate, whereas the dissolved amount of glyphosate was evidentially very low in soils Brezje and Joy A19 (lower than 1 % of the initial glyphosate).

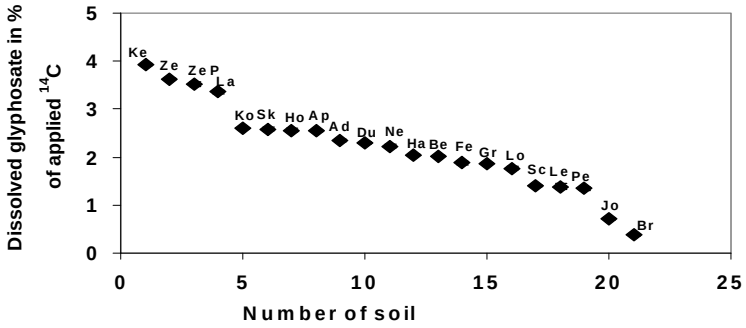


Figure 5.14. The relative glyphosate dissolution ranking of the 21 investigated soils with PW approach

Same as the adsorption of glyphosate with OECD approach, glyphosate adsorption with PW approach in soil is very high. It varies from 93 % to 100 % of the applied glyphosate. In all soils, mass balance of ^{14}C-glyphosate was quite good, over 95 % of totally applied ^{14}C-glyphosate for all soils was recovered after the sorption experiments (Table 5.4).

Table 5.4. Dissolution, adsorption and recovery of glyphosate in the 21 soils with PW approach

Soil	PW approach		
	Dissolved amount (%)[a]	**Adsorbed amount (%)[a]**	**Total recovery (%)[a]**
Ada A02	2.3 (±0.0)	93.2 (±2.4)	95.5 (±2.4)
Apace-njiva	2.6 (±0.0)	98.1 (±0.8)	100.7 (±0.8)
Berta A02	2.0 (±0.0)	94.2 (±1.9)	96.2 (±1.9)
Brezje	0.4 (±0.0)	99.8 (±2.1)	100.2 (±2.1)
Dunja A06	2.3 (±0.0)	99.7 (±0.3)	102.0 (±0.3)
Feldkirchen	1.9 (±0.0)	98.4 (±1.2)	100.2 (±1.2)
Grace A13	1.9 (±0.0)	94.7 (±5.4)	96.6 (±5.4)
Hanna A15	2.1 (±0.0)	97.7 (±0.9)	99.8 (±0.9)
Hohenwart	2.6 (±0.0)	96.4 (±1.6)	99.0 (±1.5)
Joy A19	0.7 (±0.0)	94.9 (±2.4)	95.7 (±2.4)
Kelheim	3.9 (±0.0)	93.8 (±0.8)	97.7 (±0.8)
Konjise	2.6 (±0.0)	95.7 (±1.0)	98.3 (±1.0)
Lamanose	3.4 (±0.0)	94.1 (±1.2)	97.4 (±1.2)
Lea A18	1.4 (±0.0)	100.9 (±3.1)	102.3 (±3.1)
Lomanose	1.8 (±0.0)	95.2 (±2.2)	97.0 (±2.2)
Neumarkt	2.2 (±0.0)	96.5 (±2.1)	98.7 (±2.1)
Pear A20	1.3 (±0.0)	100.0 (±1.5)	101.3 (±1.5)
Scheyern-lysi	1.4 (±0.0)	102.8 (±0.6)	104.2 (±0.6)
Skrinjar	2.6 (±0.0)	97.6 (±0.8)	100.2 (±0.8)
Zepovci	3.6 (±0.0)	93.0 (±1.4)	96.6 (±1.4)
Zepovci(Plitv.)	3.5 (±0.0)	94.5 (±1.3)	98.0 (±1.3)

a) % of applied ^{14}C-glyphosate; the mean value is presented and the values in parentheses is standard deviation

5.2.6. Relationship between dissolved glyphosate using 2 extraction approaches (OECD and PW) and mineralization at the first day

5.2.6.1. OECD approach

A univariate correlation between mineralization at the first day and dissolved glyphosate was calculated to examine whether the sorption of glyphosate will influence the mineralization of glyphosate at the first day. The results are presented in Figure 5.15. There was a significantly positive correlation ($p = 0.0002$) between mineralization of glyphosate at the first day and dissolved glyphosate in soils with OECD approach.

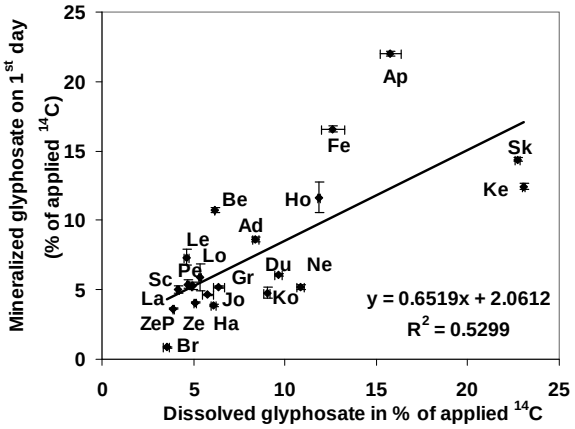


Figure 5.15. Correlation between dissolved glyphosate (OECD approach) and cumulative mineralization at the first day of the biodegradation experiments (bars indicate standard deviation)

5.2.6.2. PW approach

PW approach was expected to be a promising method for estimating the actual bioavailability of glyphosate in soil as compared to the OECD approach since OECD approach is conducted under very artificial conditions (addition of water in excess and strong shaking) which can break soil aggregates and lead to a significant increase of the available soil surface area which increasingly interacts with pesticide molecules. Whereas sorption experiments with PW approach was carried out under relatively realistic conditions like in natural soils and they were conducted under the same conditions as the mineralization experiments. However, the results which are presented in Figure 5.16 show that there is no reasonable correlation between dissolved glyphosate (PW) and mineralization at the first day.

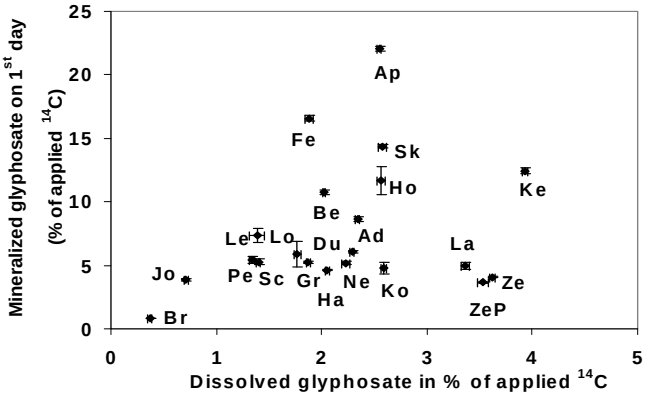


Figure 5.16. Correlation between dissolved glyphosate (PW approach) and cumulative mineralization at the first day of the biodegradation experiments (bars indicate standard deviation)

5.2.7. Effect of NaN_3 on the quality of soil pore water

The result from **5.2.6.2** shows that no correlation between mineralization at day 1 and sorption behaviour of glyphosate by PW approach was found although both experiments were conducted under the same experimental conditions. Now the question arose what could be the reason for that lacking correlation. One reason could perhaps be the high NaN_3 concentration which has adopted from the literature. Therefore, an additional *in situ* experiments with and without NaN_3 addition to soil were conducted to examinee whether high amount of applied NaN_3 artificially effects on the quality of soil pore water and sorption of glyphosate. As can be seen from Table 5.5, application of high concentration of NaN_3 (6,500 µg NaN_3 g^{-1} soil) caused a reduction of soil pH (between 0.2 and 0.5 pH units). And it turned out that the pore water of the soils with NaN_3 addition showed a reddish colour while the pore water without NaN_3 addition were not coloured (transparent). The intensity of colour increased with an increase in amount of oxalate extractable Fe^{3+} in soils. This shows clearly that in the presence of NaN_3, iron was dissolved in the soil solution by forming a complex with N^-_3 from NaN_3. Thus, sorption behaviour of glyphosate was strongly influenced by the high NaN_3 concentrations and therefore a correlation between mineralization and dissolved glyphosate could not be found. Because of this effect, the sorption results are falsified and it is not astonishing that there was no correlation between mineralisation and dissolved glyphosate by PW approach.

Table 5.5. The pH and colour of soil pore water in the treatments with and without NaN_3 application

No.	Soil	pH		Colour	
		1) No NaN_3	2) NaN_3 application	1) No NaN_3	2) NaN_3 application
1	Konjise	7.3	7.0	Transparent	Red + +
2	Zepovci(Plitv.)	6.0	5.6	Transparent	Red + +
3	Lamanose	6.2	6.0	Transparent	Red + + +
4	Zepovci	6.3	5.8	Transparent	Red + + +
5	Brezje	6.0	5.5	Transparent	Red + + + +

+ represents for colour intensity

5.2.8. Calculation of correlations between dissolved glyphosate using 2 extraction approaches (OECD and PW) and soil properties

5.2.8.1. OECD approach

A univariate correlation between dissolved glyphosate and soil parameters was calculated to examine which soil parameters govern the sorption of glyphosate in soil. The results are presented in Figure 5.17. A significantly negative correlation was found between sorption of glyphosate (OECD approach) with exchangeable H^+ ($p = 0.0000$) and clay content ($p = 0.0014$), while a positive correlation between dissolved glyphosate and soil pH ($p = 0.0001$) was observed.

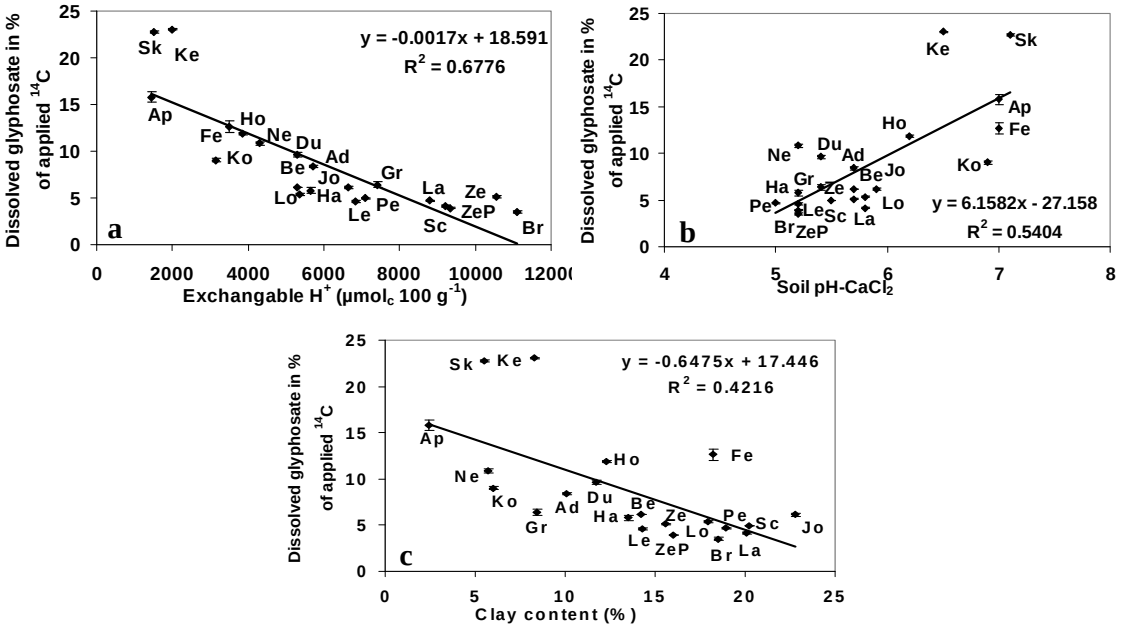


Figure 5.17. Correlations of dissolved glyphosate (OECD approach) with exchangeable H^+ (a), soil pH (b) and clay content (c) (n = 21, bars indicate standard deviation)

In order to investigate the interacting functions of the different soil parameters on dissolved glyphosate by OECD approach, a multiple regression analysis was used. The input parameters were exchangeable [H^+], silt, clay, soil organic matter, C, N, C/N, P_2O_5, Cu^{2+}, oxalate extractable Al^{3+}, oxalate extractable Fe^{3+}, K_2O, Ca^{2+}, Mg^{2+}, K^+, Na^+, CEC, pH and H^+-pH. The dissolved glyphosate (OECD) was best described by the model D (n = 21):

Dissolved glyphosate (OECD) [%] = 6.34 x pH - 4.32 x [C] – 0.08 x [Silt] – 18.37

(Adjusted $R^2 = 0.85$, $p = 7.57 \times 10^{-8}$)

[C] is organic carbon content in soil (%).

The result of multiple regressions reveals that soil pH, total carbon content and silt are the most important factors contributing collectively to dissolved glyphosate (OECD) in the 21 investigated soils.

5.2.8.2. PW approach

No reasonable correlation between dissolved glyphosate and any soil parameters was found when taking dissolved glyphosate (PW approach) and soil parameters into account for calculation of correlation.

5.3. Desorption behavior of glyphosate in soils

Desorption of glyphosate on 21 soils was studied using 2 methods: (1) the standard batch experiments with an excess of water based on OECD guideline ($OECD_{de}$ approach) and (2) soil pore water extraction approach (PW_{de}). Desorption experiments provide more information about the probability of glyphosate released to soil solution from the absorbed pool in soil matrix where it can be degraded and or leach to ground water. The aim of this study was (1) to investigate the desorbable glyphosate in soil, (2) to find out whether desorption of glyphosate regulates the mineralization of glyphosate in soil and (3) to check which soil parameters govern the desorption behavior of glyphosate.

5.3.1. $OECD_{de}$ approach

Desorption of glyphosate from 21 soils using the $OECD_{de}$ approach (modified OECD Guideline 106, refer **4.7.3**) is shown in Table 5.6. To be able to compare the correlation calculation between desorbed and mineralized glyphosate, the desorbed amount of glyphosate was calculated by basing on % of initially applied glyphosate. Desorption of glyphosate was

slight and various. Total desorbed amounts of glyphosate during the 6 successive desorption steps were varied roughly between 5.0 % and 49.0 % of the initial glyphosate. The desorption of glyphosate on soil Brezje was very low, about 5.7 % of the initial glyphosate, whereas soil Skrinjar had a total desorbed amount of 49.0 % which is the highest value measured in the 21 investigated soils. Total desorbed amount of glyphosate in other soils varied between 7.7 and 43.9 % of the initial glyphosate.

Table 5.6. Desorbed glyphosate in the 21 soils during the six desorption steps using $OECD_{de}$ approach

Soil	**Initially dissolved Gly. (%)**	% desorbed glyphosate[a)]					
		Desorbing steps					
		1 (1.67 d*)	2 (2.67 d*)	3 (3.67 d*)	4 (4.67 d*)	5 (5.76 d*)	**Total Desorbed**
Ada A02	9.3 (±0.1)	3.1 (±0.0)	2.2 (±0.0)	1.9 (±0.0)	1.5 (±0.0)	1.3 (±0.0)	19.3 (±0.1)
Apace-njiva	15.6 (±0.3)	5.2 (±0.1)	4.0 (±0.1)	3.5 (±0.1)	3.0 (±0.0)	2.7 (±0.0)	34.0 (±0.3)
Berta A02	6.7 (±0.1)	2.1 (±0.2)	1.6 (±0.0)	1.3 (±0.1)	1.1 (±0.1)	1.0 (±0.1)	13.8 (±0.4)
Brezje	3.3 (±0.1)	0.8 (±0.0)	0.6 (±0.0)	0.3 (±0.0)	0.3 (±0.0)	0.2 (±0.0)	5.5 (±0.2)
Dunja A06	7.2 (±0.3)	2.8 (±0.1)	2.3 (±0.1)	2.1 (±0.1)	1.9 (±0.1)	1.8 (±0.1)	18.0 (±0.2)
Feldkirchen	13.4 (±0.1)	4.5 (±0.0)	3.6 (±0.0)	2.9 (±0.0)	2.6 (±0.1)	2.2 (±0.0)	29.0 (±0.6)
Grace A13	6.3 (±0.3)	2.1 (±0.1)	1.7 (±0.1)	1.4 (±0.0)	1.2 (±0.0)	1.0 (±0.0)	13.7 (±0.2)
Hanna A15	5.4 (±0.2)	1.7 (±0.1)	1.4 (±0.1)	1.1 (±0.1)	1.0 (±0.1)	0.8 (±0.0)	11.4 (±0.2)
Hohenwart	10.0 (±0.2)	3.7 (±0.1)	3.2 (±0.0)	2.7 (±0.0)	2.4 (±0.1)	2.1 (±0.1)	24.2 (±0.5)
Joy A19	6.2 (±0.1)	2.1 (±0.1)	1.7 (±0.1)	1.2 (±0.0)	1.1 (±0.1)	1.0 (±0.0)	13.3 (±0.3)
Kelheim	23.0 (±0.6)	6.4 (±0.2)	4.9 (±0.1)	3.8 (±0.1)	3.2 (±0.1)	2.7 (±0.1)	43.9 (±0.8)
Konjise	9.0 (±0.2)	2.4 (±0.0)	1.9 (±0.1)	1.7 (±0.0)	1.4 (±0.0)	1.2 (±0.0)	17.6 (±0.2)
Lamanose	5.2 (±0.1)	1.1 (±0.1)	0.9 (±0.0)	0.6 (±0.0)	0.5 (±0.0)	0.4 (±0.0)	8.5 (±0.1)
Lea A18	4.7 (±0.0)	1.5 (±0.0)	1.0 (±0.0)	0.8 (±0.0)	0.7 (±0.0)	0.6 (±0.1)	9.3 (±0.1)
Lomanose	3.7 (±0.0)	1.2 (±0.0)	1.0 (±0.0)	0.9 (±0.0)	0.9 (±0.0)	0.8 (±0.0)	8.4 (±0.1)
Neumarkt	7.1 (±0.7)	2.6 (±0.2)	2.2 (±0.1)	2.0 (±0.1)	1.7 (±0.1)	1.7 (±0.1)	17.4 (±0.6)
Pear A20	4.1 (±0.2)	1.5 (±0.1)	1.1 (±0.0)	0.9 (±0.0)	0.7 (±0.0)	0.6 (±0.0)	8.9 (±0.1)
Scheyern-lysi	4.4 (±0.2)	1.7 (±0.1)	1.2 (±0.1)	1.0 (±0.1)	0.8 (±0.0)	0.7 (±0.0)	9.8 (±0.4)
Skrinjar	22.5 (±0.3)	7.8 (±0.1)	6.1 (±0.1)	4.9 (±0.1)	4.1 (±0.1)	3.4 (±0.1)	48.7 (±0.7)
Zepovci	4.6 (±0.4)	1.5 (±0.1)	1.1 (±0.1)	0.8 (±0.0)	0.8 (±0.0)	0.7 (±0.0)	9.4 (±0.1)
Zepovci(Plitv.)	4.0 (±0.1)	1.0 (±0.1)	0.8 (±0.0)	0.5 (±0.0)	0.4 (±0.0)	0.4 (±0.0)	7.0 (±0.1)

* day *a) % of applied ^{14}C-glyphosate; the mean value is presented and the values in parentheses is standard deviation.*

5.3.2. PW_{de} approach

Since desorption via the OECD approach is artificial (an excess of water, vigorous shaking) desorption via the more realistic PW approach was additionally applied. Desorption of glyphosate from 3 soils via PW_{de} approach is depicted in Table 5.7. The data showed that desorption was only slight. During the 16 successive desorption steps (about 20 days), the desorbed amount of glyphosate via PW extraction was only low. Approximately 25, 31 and 21 % of the initially applied glyphosate were desorbed in soil water from Apace-njiva, Skrinjar and Hohenwart soils, respectively.

Table 5.7. Desorbed glyphosate in the 3 soils during the sixteen desorption steps using PW_{de} approach

Desorption steps	Time for incubation (d)	Soils		
		Apace-njiva (%)[a)]	Hohenwart (%)[a)]	Skrinjar (%)[a)]
1 (dissolved amount)	1	3.5 (±0.2)	2.4 (±0.1)	3.9 (±0.1)
2	2	1.7 (±0.1)	1.0 (±0.2)	2.0 (±0.1)
3	3	1.4 (±0.1)	1.1 (±0.1)	1.7 (±0.1)
4	4	1.5 (±0.1)	1.1 (±0.0)	1.9 (±0.0)
5	5	1.5 (±0.1)	1.2 (±0.0)	2.0 (±0.0)
6	7	1.6 (±0.0)	1.3 (±0.1)	2.1 (±0.0)
7	8	1.5 (±0.1)	1.3 (±0.0)	1.8 (±0.4)
8	9	1.5 (±0.1)	1.3 (±0.0)	2.0 (±0.1)
9	10	1.5 (±0.1)	1.3 (±0.0)	2.0 (±0.0)
10	11	1.4 (±0.1)	1.3 (±0.1)	2.0 (±0.1)
11	14	1.6 (±0.1)	1.4 (±0.0)	1.9 (±0.0)
12	15	1.3 (±0.1)	1.3 (±0.1)	1.8 (±0.0)
13	16	1.3 (±0.0)	1.2 (±0.1)	1.7 (±0.0)
14	17	1.2 (±0.0)	1.2 (±0.0)	1.6 (±0.0)
15	18	1.2 (±0.0)	1.2 (±0.0)	1.6 (±0.0)
16	21	1.3 (±0.1)	1.3 (±0.0)	1.6 (±0.0)
Total desorbed amount (%)[a]		25.1	21.0	31.3

a) % of applied ^{14}C-glyphosate; the mean value is presented and the values in parentheses is standard deviation.

5.3.3. A comparison between 2 approaches regarding desorbed amount of glyphosate

A comparison of the two different desorption methods used in 3 different soils shows that the desorption rates of glyphosate with $OECD_{de}$ approach is higher than that with PW_{de}

approach (Figure 5.18). The desorption rate sharply reduced in the 2nd desorption step for all 3 soils. Afterwards the rate of desorbable glyphosate ($OECD_{de}$) declined gradually over time. With the PW approach (PW_{de}) a fair decrease of desorption rate was observed in the 2nd desorption step and thereafter the desorption rate was more or less stable over time.

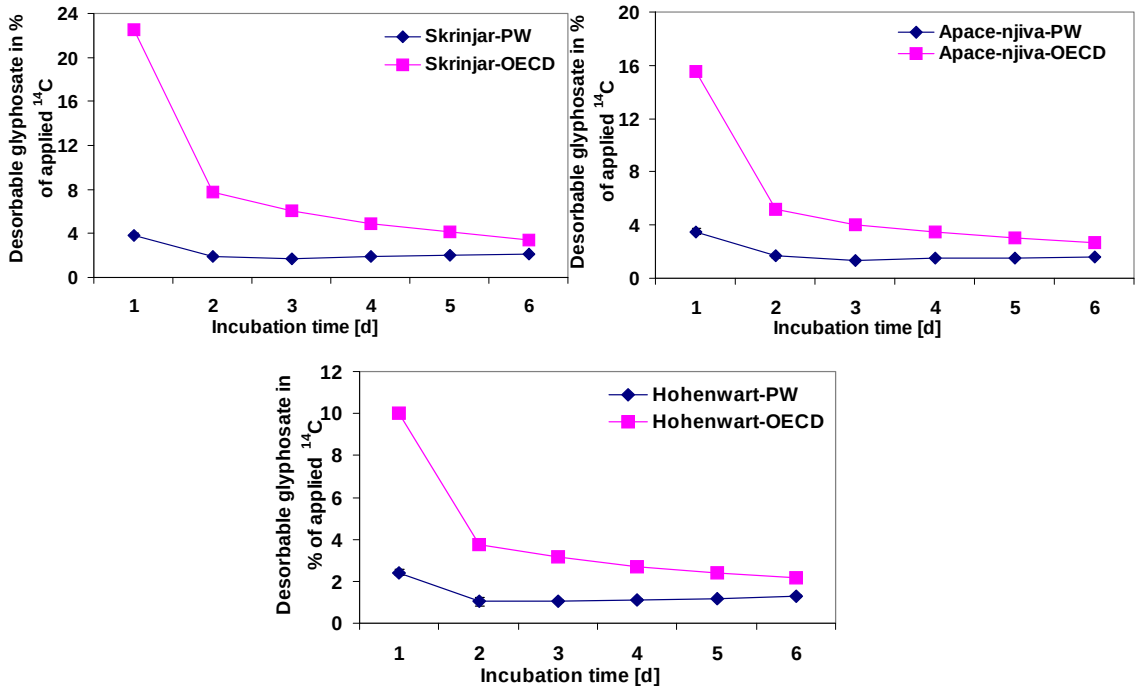


Figure 5.18. Desorbable glyphosate using 2 apporaches ($OECD_{de}$ and PW_{de}) in the 3 soils (Skrinjar, Apace-njiva and Hohenwart) during 6 desorption steps (bars indicate standard deviation)

5.3.4. A comparison between cumulative desorption ($OECD_{de}$) and cumulative mineralization of glyphosate

In this part, desorbed glyphosate by PW approach was not involved for the correlation with cumulative mineralization since only 3 soils conducted for desorption experiments. In this comparison, cumulatively desorbed glyphosate also means as dissolved amount of glyphosate in soil solution. The comparison between the cumulative mineralization and desorption of glyphosate was calculated for the mineralization after 6 days since only 6 desorption steps were conducted in desorption experiments with $OECD_{de}$ approach. The time for 6 desorption steps is relatively corresponding to 6 days in biodegradation experiments. A comparison of these 2 parameters during 6 days shows that the cumulative mineralization of glyphosate was higher than the cumulative desorption of glyphosate (Figure 5.19), except soils Skrinjar, Kelheim, Neumarkt and Brezje. The cumulative desorption in these soils was

higher than the cumulative mineralization. Additionally, the total cumulative mineralization of glyphosate was much higher than the cumulative desorption in soils Apace-njiva, Feldkirchen, Hohenwart, Konjise, Berta A02, Joy A19, Pear A20, and Lomanose while in other soils the cumulative mineralization was slightly higher than the cumulative desorption of glyphosate.

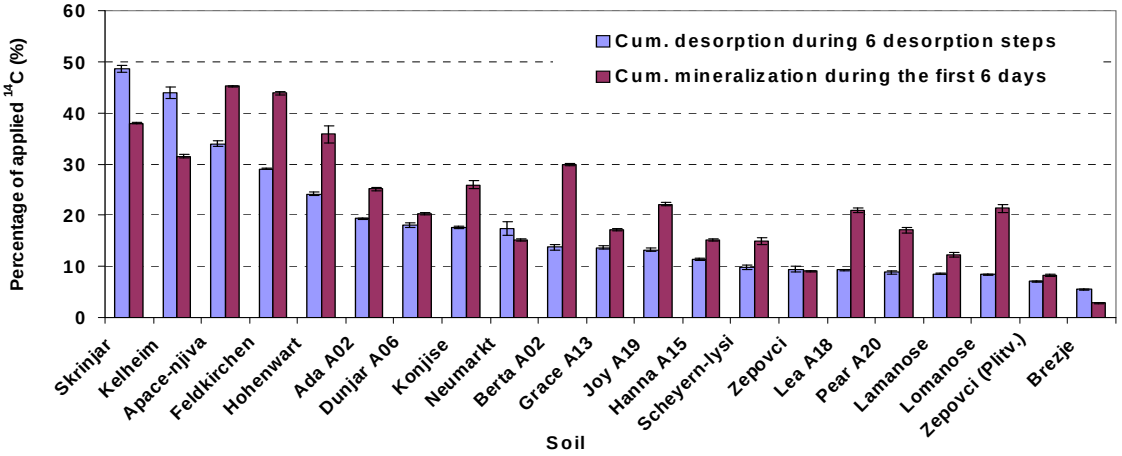


Figure 5.19. Cumulative desorption and mineralization of glyphosate measured in percentage of the applied amount of glyphosate in course of 6 day incubation (bars indicate standard deviation).

There was a significantly positive correlation between the cumulative mineralization and desorption of glyphosate ($p = 0.0000$) (Figure 5.20). This means that the more glyphosate is desorbed, the more glyphosate is mineralized in most of the soils, but this phenomenon is not suitable for soils: Skrinjar, Kelheim, Neumarkt and Brezje since these soils have higher glyphosate desorbed than glyphosate mineralized. Therefore, these 4 soils were not included in univariate correlation between cumulative mineralization and desorption of glyphosate. All in all, the results allow concluding that desorption is also an important factor contributing on the mineralization of glyphosate in soils. But it is astonishing, that more glyphosate was mineralized than was dissolved in soil solution. Therefore, additionally experiments about uptake of glyphosate by the microbial cell should be conducted to explain this discrepancy.

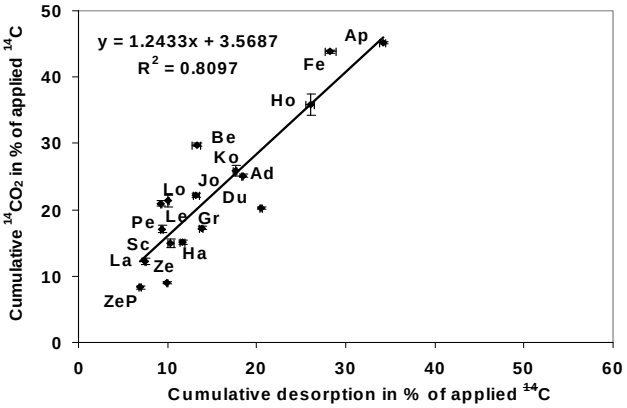


Figure 5.20. Correlation between cumulative mineralization in course of 6 day incubation and cumulative desorption of glyphosate within 6 desorption steps ($OECD_{de}$ approach) on the 17 soils (bars indicate standard deviation)

5.3.5. Calculation of correlations between desorption of glyphosate (OECD) and soil properties

In this part, desorbed glyphosate by PW approach was not involved for the correlation with cumulative mineralization since only 3 soils conducted for desorption experiments. Since one of the main objectives of this study was to identify the soil factors which govern glyphosate desorption, several univariate correlations and a multivariate correlation between cumulative mineralization and soil parameters were calculated. The results of univariate correlation are presented in Figure 5.21. The desorbed amounts of glyphosate was negatively correlated to exchangeable H^+ (p = 0.0000), but positively correlated with soil pH (p = 0.0000). This means that the desorbed amount of glyphosate was strongly influenced by soil exchangeable H^+, and soil pH.

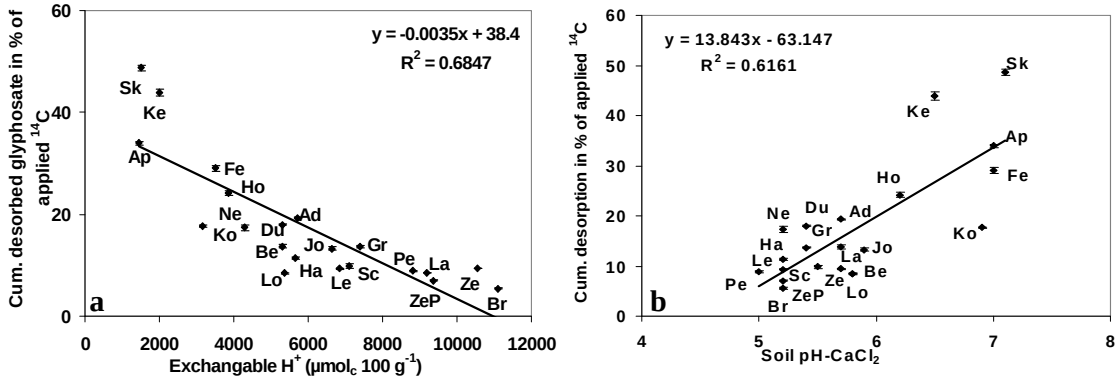


Figure 5.21. Relationships of cumulative desorption of glyphosate within 6 desorption steps ($OECD_{de}$ approach) with exchangeable H^+ (a), and soil pH (b) (bars indicate standard deviation)

In order to investigate the interacting functions of the different soil parameters on cumulative desorbed glyphosate (6 steps) using OECD approach, a multiple regression analysis was used. The input parameters were exchangeable [H^+], silt, clay, soil organic matter, C, N, C/N, P_2O_5, Cu^{2+}, oxalate extractable Al^{3+}, oxalate extractable Fe^{3+}, K_2O, Ca^{2+}, Mg^{2+}, K^+, Na^+, CEC, pH and [H+]-pH. The cumulative desorbed glyphosate (6 steps) using OECD was best described by the model E (n = 21):

$$\text{Cumulative desorbed glyphosate in 6 steps (OECD) [\%]} = -0.001 \times [H^+_{Exc.}] + 14.176 \times pH - 5.227 \times [Mg^{2+}] - 49.607$$

(Adjusted $R^2 = 0.87$, $P = 0.000$)

$[H^+_{Exc.}]$ is exchangeable H^+ (%)

The result of multiple regressions reveals that soil exchangeable H^+, pH, and $Mg^{2+}$are the most important factors contributing collectively to desorption of glyphosate (OECD) in the 21 tested soils.

5.4. Quantification of glyphosate in soil pore solution shortly after application

The aim of this experiment was (1) to study the dynamics of ´´real bioavailability´´ and dissolution of glyphosate in soil pore solution shortly after application to clarify the question, whether the microorganisms influent on the sorption process of glyphosate in soil and (2) to compare the mineralization rate of glyphosate per cell in soil and nutrient solution.

5.4.1. Mineralization of glyphosate in soil Feldkirchen within 3 days

The aim of this experiment was to measure the real bioavailability of glyphosate in soil shortly after application of glyphosate in condition of with and without involvement of soil microbes in one selected soil. Soil Feldkirchen was selected because the mineralization of glyphosate was very high and the mineralized glyphosate was much higher than desorbed glyphosate. Under condition of no soil microbe involvement, NaN_3 was needed to apply to soil. After 0.17, 0.33, 0.67, 1, 2, 3 days the mineralization and dissolution in soil pore solution of glyphosate were measured. Mineralization of glyphosate in soil Feldkirchen without NaN_3 application within 3 days is shown in Figure 5.22a. There was a considerable amount of glyphosate mineralization within 3 days. Approximately 30 % of the totally applied ^{14}C-glyphosate was mineralized to $^{14}CO_2$. According to the Figure 5.22b, the mineralization rate of glyphosate declined over time. The rate of mineralization was considerably reduced during

the first 1 day and afterwards, mineralization rate reduced gradually. Conversely, almost no mineralization was observed in the biodegradation experiment with NaN_3. Within 3 days, only negligible amount, 0.04 % of the totally applied ^{14}C-glyphosate was mineralized to $^{14}CO_2$ (Figure 5.23).

Bacterial cell counts increased slightly, but significantly during the first day, and then they were stable thereafter (Figure 5.24). The significant increase of bacterial cells on the first day seems to be a result of a priming effect caused by the mixing step when applying glyphosate into soil. This mixing step makes nutrient, especially organic carbon more available for soil microbes and oxygen as well.

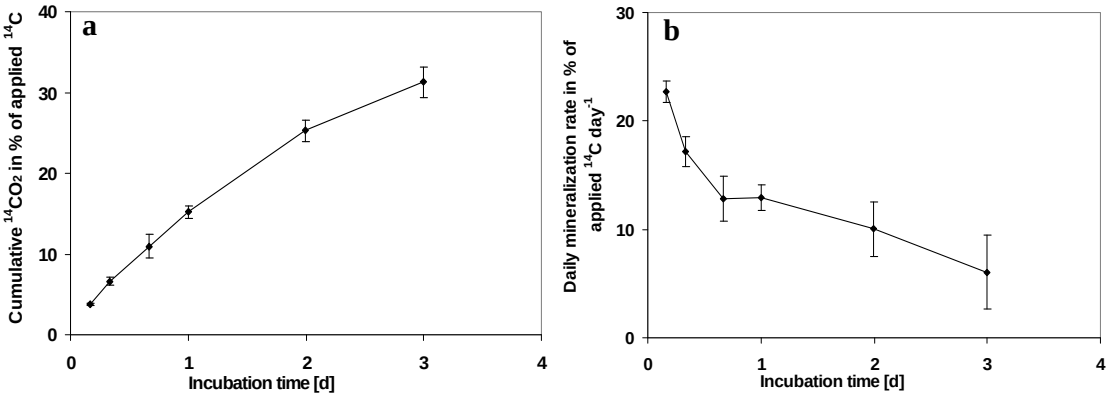


Figure 5.22. Development of cumulative mineralization (a) and mineralization rate of glyphosate (b) in soil Feldkirchen without NaN3 application (bars indicate standard deviation)

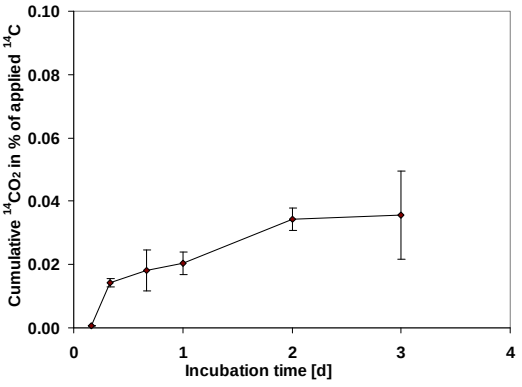


Figure 5.23. Development of cumulative mineralization of glyphosate in soil Feldkirchen with NaN_3 application (bars indicate standard deviation)

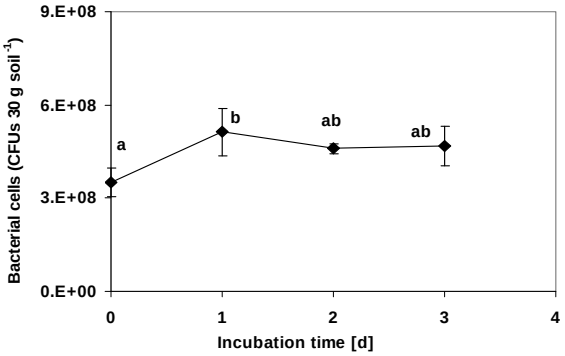


Figure 5.24. Development of bacterial cell counts in soil biodegradation experiment without NaN3 in course of 3 day incubation, the letters express the statistical differences between sampling times [bars indicate standard deviation. Values with the same letter assigned are not significantly different ($\alpha = 0.05$) according to one way Anova and Tukey HSD test].

5.4.2. Glyphosate in soil pore solution under biotic (without NaN_3) and abiotic (with NaN_3) conditions

The dissolved amount of glyphosate in soil pore solution during the biodegradation experiment without NaN_3 was quite low, but still an important portion of glyphosate in soil pore water. About 1.83 % of the applied ^{14}C was extracted in soil pore solution after 0.17 day incubation (Figure 5.25). It significantly reduced over time. During the first 0.67 day of the incubation period, the dissolved amount strongly decreased and slowly declined thereafter. After 3 days of the experimental course, only 0.43 % of the totally applied glyphosate was detected in soil pore solution. The dissolved glyphosate in soil pore water is a small part of the ´´*in situ*´´ desorption portion. There was a significant difference concerning the dissolved amount of glyphosate between sampling points of 0.17 day, 0.33 day and 0.67 day (Figure 5.25). Additionally, no difference was found between the sampling points of 0.67 day and 1 days, 2 days and 3 days concerning glyphosate concentration in pore water. *In situ* bioavailability values and the values of mineralization rates (Figure 5.22b) during the biodegradation experiment without NaN_3 application had the same pattern and behavior. This clearly indicates that the mineralization rate of glyphosate in Feldkirchen soil has a strong relation to the bioavailability of glyphosate in soil pore solution and ´´in situ´´ desorption of glyphosate in soil pore solution must be relatively high.

As also observed in Figure 5.25, the dissolved amounts of glyphosate in soil pore solution at several sampling points during the biodegradation experiment with NaN_3 application were not very much higher than that in the biodegradation without NaN_3 and

varied between 2.60 and 2.92 % of the totally applied ^{14}C. Under abiotic conditions with NaN_3, the sorption equilibrium of glyphosate was reached very rapidly, just after 0.17 day. No significant difference regarding the dissolved amount was found between sampling times. A comparison of mineralized amount (in biotic condition) and dissolved amount of glyphosate (in abiotic condition) shows that the mineralized amount of glyphosate during 0.17 day (3.78 %) was significantly higher than the dissolved amount (2.92 %) under abiotic conditions (α = 0.05, Tukey HSD tests). This indicates that under abiotic conditions, glyphosate was adsorbed to soil matrix very rapidly whenever it was introduced to soil. Microbial community, perhaps, plays an essential role in retarding the glyphosate adsorption on soil matrix. The microbes may compete with soils for ´´uptake´´ of glyphosate. Therefore, the conclusion can be drawn from the results of this experiment is that a difference in term of bioavailability of glyphosate between biotic and abiotic conditions is caused by soil microbes.

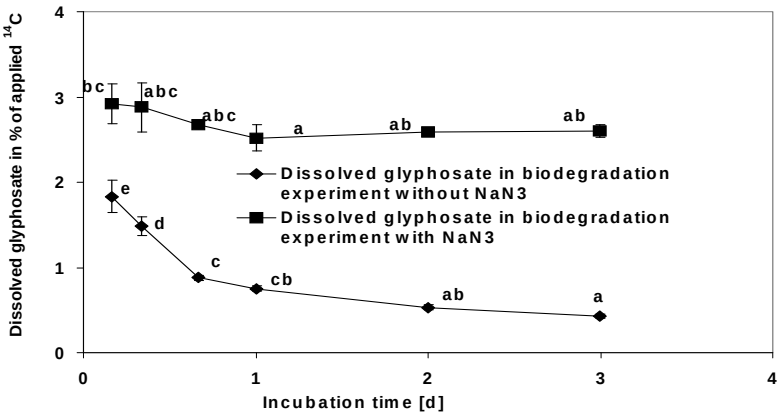


Figure 5.25. Development of dissolved amount of glyphosate in soil pore water in biodegradation experiments with and without NaN_3, the letters express the statistical differences between sampling times [bars indicate standard deviation. Values with the same letter assigned are not significantly different (α = 0.05) according to one way Anova and Tukey HSD test]

Determination of desorbed amount of glyphosate was carried out after the biodegradation experiment with NaN_3 using the soil pore water extraction approach. After the first extraction for determining the *in situ* bioavailability of glyphosate in biodegradation experiment with NaN_3 application, *in situ* desorption of glyphosate was determined after 1, 3, 4, 13, 14 and 15 hours. The results are presented in Figure 5.26. Roughly 0.15; 0.33; 0.32; 0.23; 0.23 and 0.26 % of the initially applied glyphosate was desorbed after 1, 3, 4, 13, 14 and 15 hour incubations, respectively. Desorbed amount of glyphosate during 4 hours (0.32 %) was much lower than the mineralized amount during 4 hours (2.86 % of the initial glyphosate

was mineralized from hours 4 to 8 in the biodegradation experiment without NaN_3 application). Therefore, the conclusion can be drawn from these results that microorganisms mainly cause desorption of glyphosate in soil pore solution.

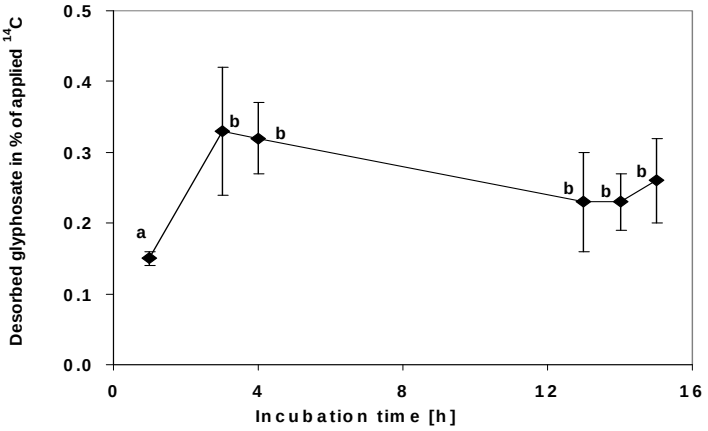


Figure 5.26. Desorbed amount of glyphosate in Feldkirchen soil, the letters express the statistical differences between sampling times [bars indicate standard deviation. Values with the same letter assigned are not significantly different (α= 0.05) according to one way Anova and Tukey HSD test].

5.5. Glyphosate biomineralization capacity of the soil microbial community in nutrient solution

The results of the biodegradation experiments (**5.1.1**) show that a high amount of glyphosate was initially mineralized at the first day for all 21 soils. This means that bioavailability of glyphosate is very high shortly after application and that glyphosate mineralization in soils is mainly regulated by its bioavailability. Therefore, this experiment (**4.5**) was conducted to measure the maximum biomineralization capacity of a soil microbial community shortly after application in an environment where the microbial activity is not limited by sorption processes to check the above mentioned hypothesis: ´´with a relatively similar microbial community, the mineralization of glyphosate in nutrient solution will be higher than that in soil as in nutrient solution, glyphosate is free for degradation by microbes and not limited by sorption processes´´.

5.5.1. Growth of the bacteria in nutrient solution during the experiment

After glyphosate herbicide was spiked to the medium, microbes in all treatments grew rapidly to maximum CFU numbers after 3 days (Figure 5.27). Thereafter, the CFU numbers decreased continuously until the end of the experiment (after 6 days).

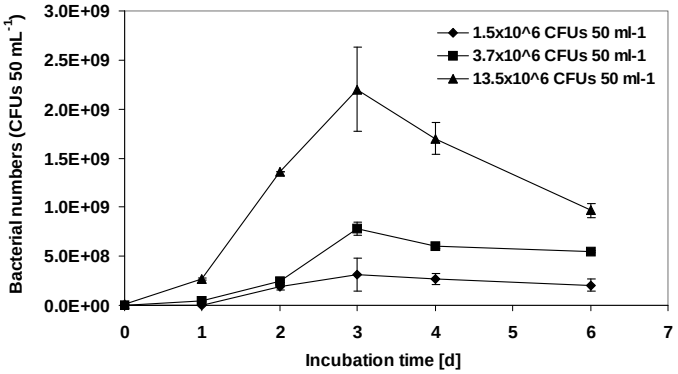


Figure 5.27. Bacterial growth dynamic during the nutrient solution experiments with different initial CFU numbers (bars indicate standard deviation)

5.5.2. Ability of the microbes to mineralize glyphosate in nutrient solution

The glyphosate degradation capacity in nutrient solution of microbes extracted from soil Feldkirchen is presented in Figure 5.28. The results show that after an incubation period of 6 days the mineralization of glyphosate was strongly dependent on the initially applied CFU numbers. At the highest applied CFU numbers (13,486,500 CFU 50 mL^{-1}) the cumulative mineralization of glyphosate was very high: 42.67 % of the applied ^{14}C-glyphosate were mineralized to $^{14}CO_2$. In the treatments with middle (3,746,250 CFU 50 mL^{-1}) and low (1,498,500 CFU 50 mL^{-1}) CFU numbers the cumulative mineralization was 4.03 % and 1.80 %, respectively.

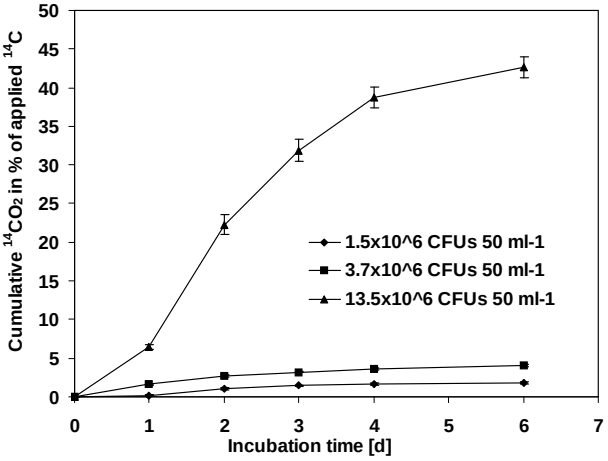


Figure 5.28. Development of cumulative mineralization of ^{14}C-glyphosate in nutrient solution with 3 different CFU numbers (bars indicate standard deviation)

5.5.3. Daily mineralization rate of glyphosate by microbes in nutrient solution

Figure 5.29 depicts the daily mineralization rates of glyphosate in nutrient solution over a period of 6 days. In the treatments initially incubated with low and high CFU numbers the maximum mineralization rate was achieved at day 2 while the maximum mineralization rate in the treatment with middle CFU numbers occurred at day 1, followed by a continuous decrease in the mineralization rate until the end of the experiment.

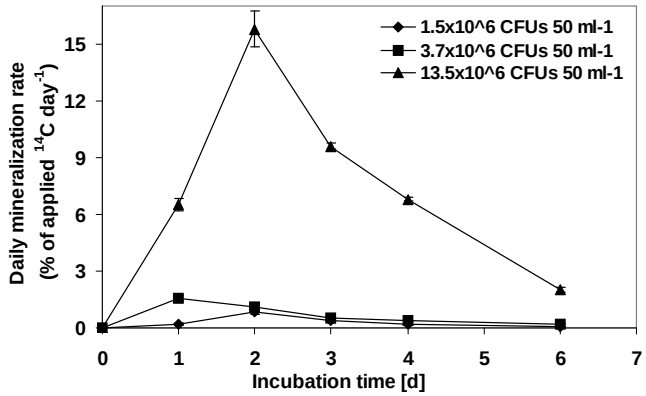


Figure 5.29. Development of daily mineralization rate of ^{14}C-glyphosate (% of applied ^{14}C day^{-1}) in nutrient solution with 3 different bacterial cell numbers (bars indicate standard deviation)

5.5.4. Daily mineralization rate of glyphosate per CFU (µg glyphosate CFU^{-1} day^{-1}) in nutrient solution

Figure 5.30 shows the daily mineralization rate of glyphosate per CFU (µg glyphosate CFU^{-1} day^{-1}) in 3 different CFU concentration treatments. At the first day the maximum mineralization rates of glyphosate per CFU were achieved in all treatments. Afterwards, a continuous decrease in mineralization rate of glyphosate was observed. In the treatment with low CFU numbers the highest mineralization rate per CFU was found whereas in the treatment with high CFU numbers the lowest mineralization rate per CFU was observed. After the first day, the mineralization rates in the middle and low bacterial concentration treatments were almost identical and lower than that in the high bacterial treatment.

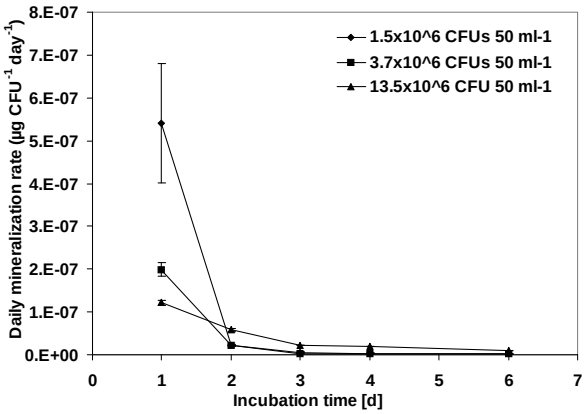


Figure 5.30. Development of daily mineralization rate (µg glyphosate CFU^{-1} day^{-1}) of ^{14}C-glyphosate in nutrient solution with 3 different CFU numbers (bars indicate standard deviation)

5.5.5. A comparison of glyphosate biodegradation capacity in soil and nutrient solution

The main aim of this part is to compare the biodegradation capacity of glyphosate by the relatively same microbial community in soil (**5.4**) and nutrient solution (**5.5**) to see whether the mineralization of glyphosate will be enhanced in nutrient solution which has no sorption sites for glyphosate or not. The result for the mineralization of glyphosate in soil and nutrient solution is depicted in Figure 5.31. In general, the cumulative mineralization of glyphosate in soil was significantly higher than in the nutrient solution, however, on the 3rd day, there was no significant difference between glyphosate mineralization in soil and in the nutrient solution with the highest CFU treatment.

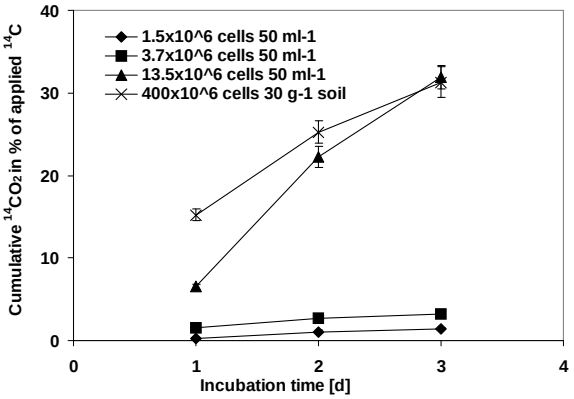


Figure 5.31. Development of short time cumulative mineralization of glyphosate in 30 g Feldkirchen soil (-x-) and in nutrient solution media with 3 microbial concentrations (bars indicate standard deviation)

Additionally, according to Figure 5.32, the daily mineralization rate of glyphosate in soil was reduced over time during 3 days of the experiment while the mineralization rate in the

nutrient solution medium mostly increased during the first 2 days and the peak was achieved at day 2 then declined afterwards.

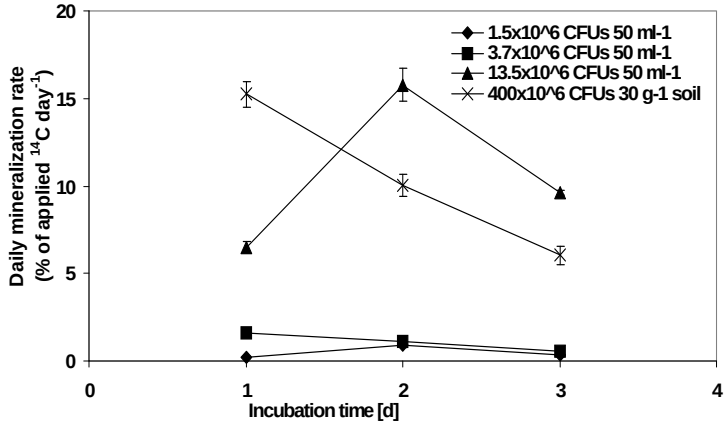


Figure 5.32. Development of daily mineralization rate of glyphosate in Feldkirchen soil (-x-) and in nutrient solution media with 3 microbial concentrations (bars indicate standard deviation)

Figure 5.33 shows daily mineralization rate of glyphosate per CFU in soil and in nutrient solution medium. The result reveals that regardless of kind of media (soil or nutrient solution) the mineralization rate per CFU was highest at day 1 and declined over the experimental time. A considerable decline in mineralization rate was found in the nutrient solution which had low and middle microbial concentration treatments from day 1 to day 2. Significant differences between treatments were found on day 1 and day 3 when making a multiple comparison among treatments. At day 1 the nutrient solution with the lowest applied CFUs showed the highest mineralization rate per CFU while the lowest mineralization rate per CFU was observed in soil. At day 2 the mineralization rate per CFU in both nutrient solutions with low and middle CFU treatments was not significantly different from each other, but both were significantly different from the soil and the nutrient solution with high CFU numbers. However, the mineralization rates per CFU at the third day were totally reversed in comparison to that at day 1. The significant differences between treatments at day 3 can be ordered as follows: nutrient solution with initially received $1.5x10^6$ CFUs < nutrient solution with initially received $3.7x10^6$ CFUs < nutrient solution with initially received $13.5x10^6$ CFUs < soil with initial $400x10^6$ CFUs.

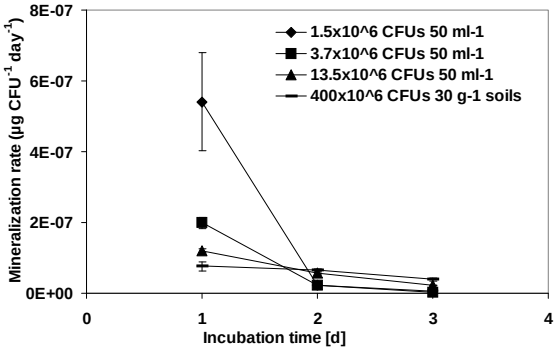


Figure 5.33. Development of mineralization rate of glyphosate per CFU (µg glyphosate day^{-1} CFU^{-1}) in 30 g Feldkirchen soil and in nutrient solution media with 3 different concentrations of microbes (bars indicate standard deviation)

The bacterial cell counts in soil and nutrient solution are shown in Figure 5.34. At the beginning of the experiment, the bacterial CFUs were higher in the soil than in the nutrient solution media. And therefore, they can take up glyphosate to a higher extent than the CFUs in the nutrient solution. The bacterial cells slightly increased until day 1 and were significantly different to the cell counts at the beginning of the experiment in all treatments. Thereafter the bacterial CFU in soil treatment was stable until day 3 while the bacterial CFUs in the nutrient solution treatments considerably gained during the first 3 days of the experiments, especially in the case of the treatment with the highest CFUs applied. At the third day of the experiment the bacterial cells in both middle and high applied CFU treatments were 8.0 x 10^8 and 2.0 x 10^9 CFUs in 50 ml, respectively while the bacterial cells were 5.0 x 10^8 CFUs in 30 g soil.

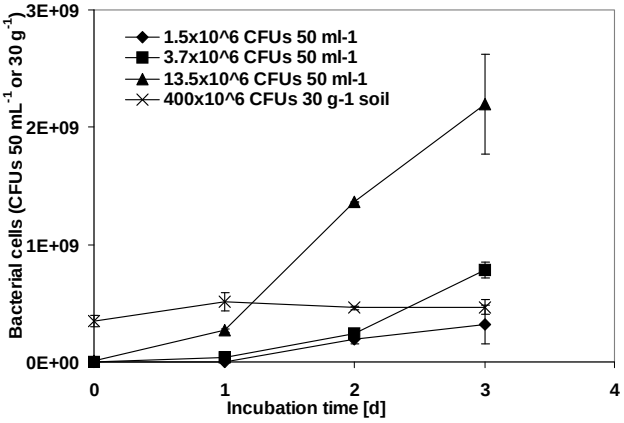


Figure 5.34. Development of bacterial cell counts in soil Feldkirchen and in nutrient solution media with 3 microbial concentrations (bars indicate standard deviation)

5.6. Dynamics of glyphosate biomineralization and glyphosate uptake by soil microorganisms in nutrient solution

The results from mineralization and desorption experiments (**5.3.4**) show that the mineralized amount of glyphosate was higher than the dissolved amount. This is astonishing. It seems that glyphosate can be taken up in a large amount by soil bacteria shortly after application and this incorporated glyphosate is further mineralized to CO_2 with time. Therefore an experiment was conducted to elucidate this process. The aim of this experiment was to investigate the dynamics of glyphosate biomineralization and glyphosate uptake by microbial cells in nutrient solution shortly after application and to clarify whether the ^{14}C-glyphosate that was taken up in the first nutrient solution is mineralized in the second nutrient solution. Therefore, two nutrient solution experiments were conducted. ^{14}C-glyphosate biomineralization was monitored in nutrient solution (phase I) in short time intervals, the microbial biomass was harvested from the nutrient solution, transferred to a new nutrient solution (phase II) with non labeled glyphosate and the production of $^{14}CO_2$ was measured.

5.6.1. Nutrient solution phase I: Glyphosate biomineralization and harvesting of microbial cells

The main aim of this experiment was to check the glyphosate uptake by microorganisms in nutrient solution over a time period of 3 days. During the experimental period the microbes were harvested at three sampling times (after 0.17, 1 and 3 days). Parallel, the mineralization of ^{14}C-glyphosate was recorded at 0.17, 1, 2 and 3 days (Figure 5.35). The initial bacterial CFUs applied to 50 mL nutrient solution were 3.5 x 10^6 CFUs. After applying ^{14}C-glyphosate to the medium, 0.1 %, 1.1 % and 3.9 % of the applied ^{14}C-glyphosate were mineralized to $^{14}CO_2$ within 0.17 day, 1 day, and 3 days, respectively (Figure 5.35a). The daily mineralization rate of glyphosate increased quickly and reached a maximum on day 1 of 1.4 % of applied ^{14}C day^{-1}. Between day 1 and day 2 the mineralization rate kept almost stable and then decreased slightly until day 3 (Figure 5.35b).

The radioactivity which remained in cell free nutrient solution after harvesting microbial cells is presented in Table 5.8. The remaining radioactivity in nutrient solution was 97.2 %, 94.6 % and 91.6 % after 0.17, 1 and 3 days, respectively.

Table 5.8. Distribution of ^{14}C-glyphosate in nutrient solution essays (phase I and II)

I. Nutrient solution phase I*	**0.17 day**	**1 day**	**3 days**
1) Mineralized amount (%)	0.1 (±0.0)	1.1 (±0.2)	3.9 (±0.2)
2) Radioactivity in free cell nutrient solution (%)	97.2 (±0.1)	94.6 (±0.4)	91.6 (±0.6)
II. Nutrient solution phase II*	**22 days**	**21 days**	**19 days**
3) Mineralized amount (%)	0.3 (±0.3)	0.5 (±0.2)	1.0 (±0.2)
4) Radioactivity on microbial cells (%)	0.6 (±0.1)	0.7 (±0.2)	0.4 (±0.1)
5) Radioactivity in free cell nutrient solution (%)	1.5 (±0.3)	2.2 (±0.2)	2.5 (±0.2)
III. Uptake ^{14}C-glyphosate after phase I (%) = 3) + 4) + 5)	**2.4 (±0.1)**	**3.4 (±0.3)**	**3.9 (±0.3)**
IV. Total mineralized glyphosate after 2 phases (%) = 1) + 3)	**0.4 (±0.3)**	**1.6 (±0.1)**	**4.9 (±0.1)**
V. Total recovery (%) = 1) + 2) + 3) + 4) + 5)	**99.7 (±0.2)**	**99.1 (±0.2)**	**99.4 (±0.7)**

**) % of applied ^{14}C-glyphosate; the mean value is presented and the values in parentheses is standard deviation.*

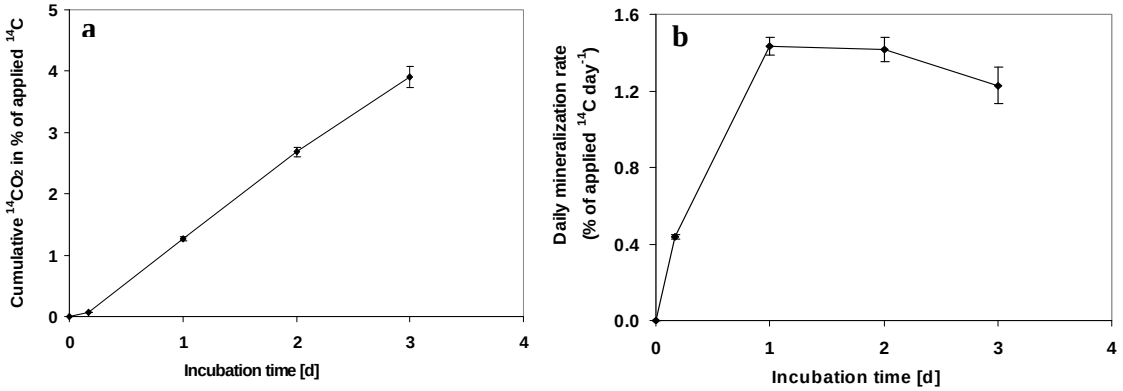


Figure 5.35. Developments of short time cumulative mineralization (a) and daily mineralization rate (b) of ^{14}C-glyphosate in the first nutrient solution phase with an initial microbial concentration of $3.5x10^6$ CFUs 50 mL^{-1} (bars indicate standard deviation)

5.6.2. Nutrient solution phase II: Biomineralization of glyphosate that was taken up by the microbial cells during nutrient solution phase I

The main objective of this experiment was to examine how fast the glyphosate that was taken up by microorganisms in the nutrient solution phase I can be degraded when the microbes are transferred to a fresh nutrient solution (phase II). Therefore, microbial cells which took up ^{14}C-glyphosate from the phase I were harvested according to the sampling times (after 0.17, 1 and 3 days) and transferred to the nutrient solution phase II containing 10 µg non-labeled glyphosate mL^{-1} and there the cells were incubated for 22, 21 and 19 days, respectively. During the experimental time the mineralization of ^{14}C-glyphosate that was taken up and incorporated in the microbial cells was observed. At the end of the experiment, radioactivity of the microbial cells and in cell free nutrient solution was measured. Finally, the

total glyphosate that was taken up by microbial cells in nutrient solution phase I was calculated as follows: mineralized ^{14}C-glyphosate in phase II ($^{14}CO_2$) + ^{14}C-glyphosate in microbial cells (determined by combustion at the end of the nutrient solution phase II) + ^{14}C-glyphosate in cell free nutrient solution at the end of the nutrient solution phase II.

The mineralization of ^{14}C-glyphosate taken up by microbial cells is shown in Figure 5.36. After 22 days, 21 days, and 19 days of incubation in nutrient solution II, 0.3 %, 0.5 %, and 1 % of the initially applied ^{14}C-glyphosate were mineralized to $^{14}CO_2$, respectively. That corresponded to 12.5 %, 14.7 % and 25.6 % of ^{14}C- glyphosate that was taken up by the microbial cells in nutrient solution phase I.

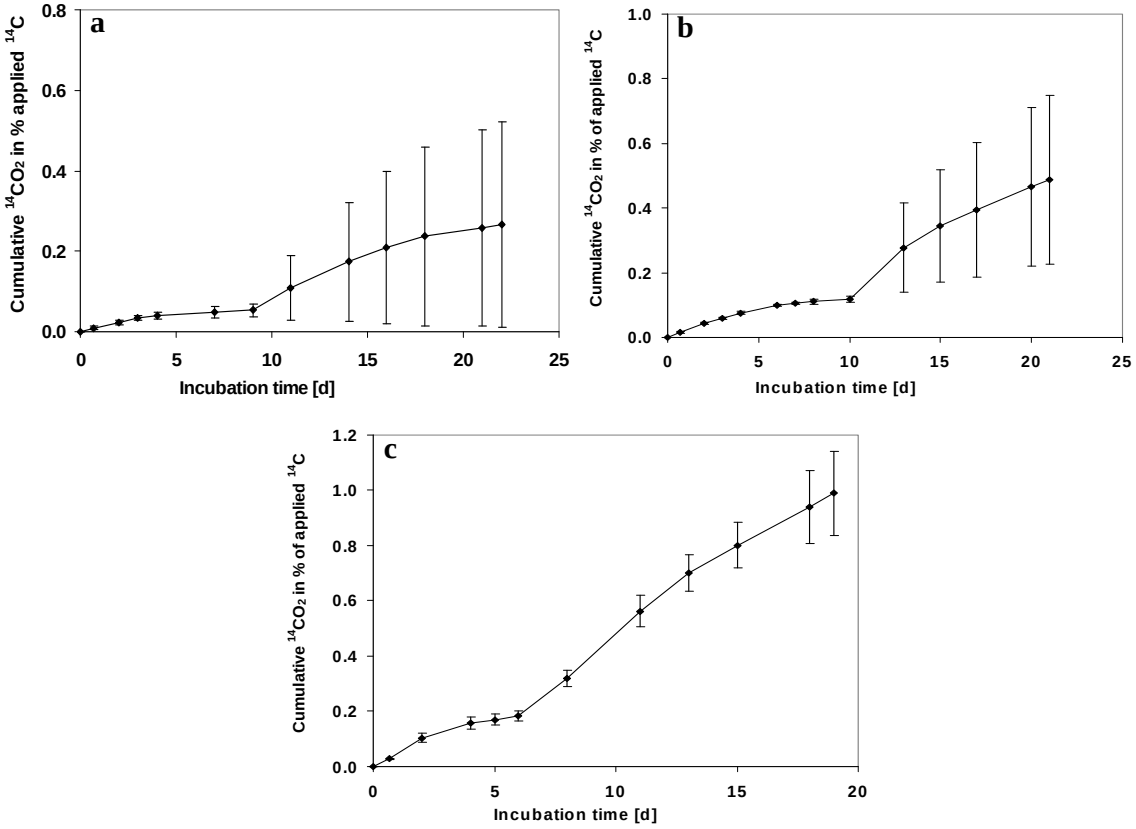


Figure 5.36. Development of mineralization of ^{14}C-glyphosate in nutrient solution phase II: microbial cells which have taken up ^{14}C-glyphosate in nutrient solution phase I were transferred to nutrient solution phase II after 0.17 day (a), 1 day (b) and 3 days (c) (bars indicate standard deviation)

5.6.3. Total uptake of glyphosate during nutrient solution phase I and recovery of radioactivity after two nutrient solution phases

Glyphosate which was taken up by microbial cells from the phase I was determined as described in **5.6.2**. Total uptake amount of glyphosate by microbial cells after 0.17 day, 1 day and 3 days from the nutrient solution phase I was 2.4 %, 3.4 %, and 3.9 % of the total applied glyphosate, respectively and is shown in Table 5.8. This indicates that with increasing incubation time the amount of ^{14}C-glyphosate that was taken up increased. This is due to an increase of bacterial CFUs during the first 3 days of the incubation period. This fact was proven in another earlier experiment that was conducted in the same manner as this experiment (**5.5.1**). A comparison between uptake and mineralization of glyphosate shows that the uptake of glyphosate by microbial cells happened very fast shortly after application, but the glyphosate that was taken up was mineralized in a longer time (Table 5.8). The radioactivity recovery was controlled after the nutrient solution phase II. The results show that the total recovery of radioactivity was quite good. Between 99.0 and 99.7 of the initially applied ^{14}C were recovered after 2 nutrient solution phases (Table 5.8).

All in all, the results of this experiment allow concluding that bacteria have the capacity to take up glyphosate in nutrient solution, but the mineralized amount of glyphosate is much lower than the amount that is taken up and the large amount of glyphosate taken up by microbes in the beginning can be mineralized in a longer time (Table 5.8).

5.7. Effect of the herbicide glyphosate on soil respiration

Soil respiration, determined as CO_2 evolution during 32 days, is demonstrated in Figures 5.37a and 5.37b. Soil respiration rate was fairly various in the 21 soils. In general, it was highest at day 1 and reduced over the incubation time. At day 1 the soil respiration rate of 21 soils varied between 47 and 206 µg CO_2 g soil^{-1} day^{-1}. The lowest and the highest soil respiration rates were found in Konjise and Feldkirchen soils, respectively. A strong decrease of soil respiration rate was found for all 21 soils from day 1 to day 2, except for Konjise soil. In this soil the soil respiration rate was more or less stable during the first 2 days (48 µg CO_2 g soil^{-1} day^{-1}) and then like in the other soils, the respiration reduced after day 2. The same pattern was found for the mineralization rate in this soil. A fair decrease of soil respiration in all 21 soils was observed between day 2 and day 6. After day 6 the soil respiration rate in all 21 soils was slightly declined over time until the end of the experiment. There were some missing values for some soils during the experiment caused by technical problems.

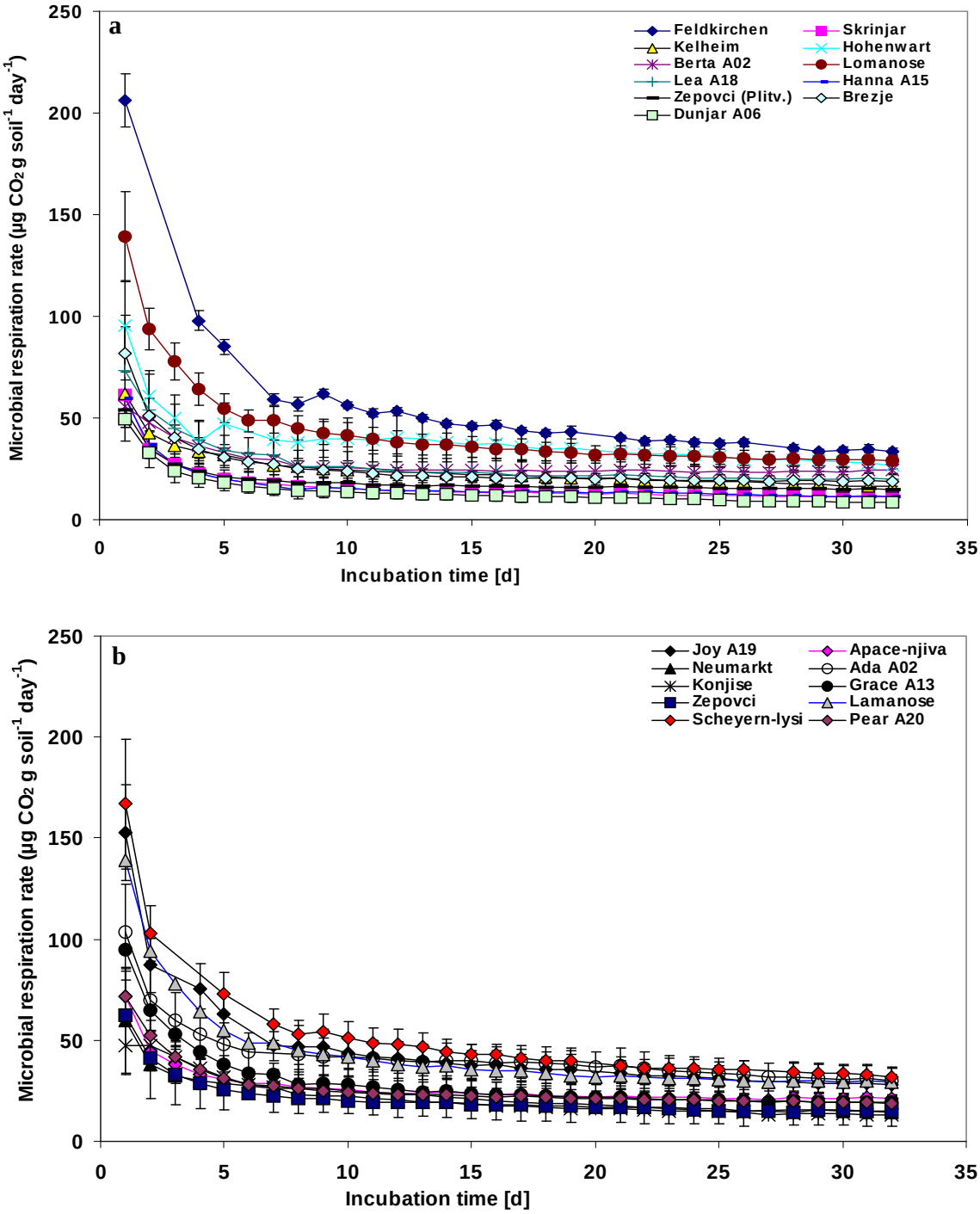


Figure 5.37. Development of microbial respiration in 21 agricultural soils applied with 10 µg glyphosate g^{-1} soil in course of 32 day incubation (bars indicate standard deviation)

Though soil respiration rates and glyphosate mineralization rates were measured under the same experimental conditions (10µg glyphosate g^{-1} soil, soil density of 1.3 g cm^{-3}, soil water tension of -150 kPa and temperature of 20 ± 1 °C) there exists no correlation between respiration and mineralization rates (Figure 5.38).

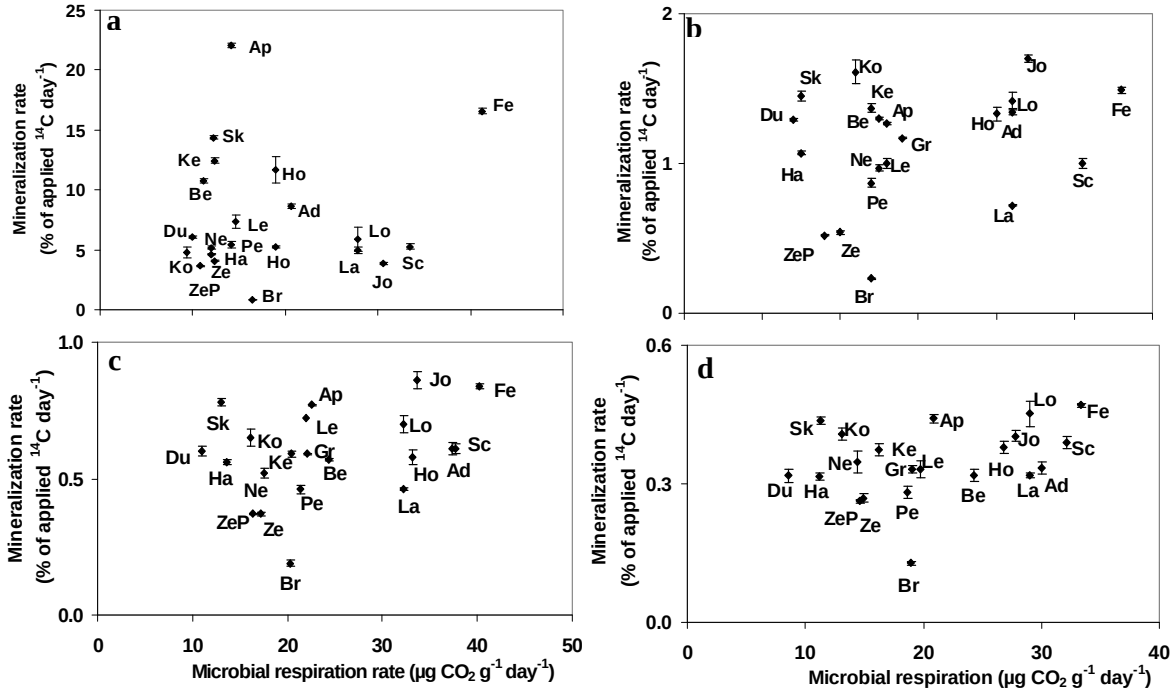


Figure 5.38. Relationships between mineralization rate and microbial respiration rate at day 1 (a), 11 (b), 20 (c) and 32 (d) (bars indicate standard deviation).

In order to assess the effect of glyphosate application on the soil respiration at the recommended pesticide dose for agricultural fields, a control treatment applying no glyphosate, but under the same experimental conditions like the glyphosate application treatment was established for each soil. After an incubation period of 32 days, the results showed that the effect of glyphosate can be divided into 3 groups: stimulating, depressing and no effects of glyphosate on soil respiration.

As presented in Figure 5.39, a significant stimulating effect of glyphosate was propounded in Lomanose, Kelheim, Neumarkt, Pear A20 and Feldkirchen soils. In these soils the soil respiration rates were significantly higher in the variants with glyphosate treatment than in the variants without glyphosate. (Mean value comparison, One-way Anova test, significant level, $\alpha = 0.05$). In Pear A20 and Feldkirchen soils, the stimulating effect of glyphosate on the soil respiration was found during the first 4 days and the first day, respectively. Afterwards no difference between the two treatments was observed (Figures 5.39d and 5.39e).

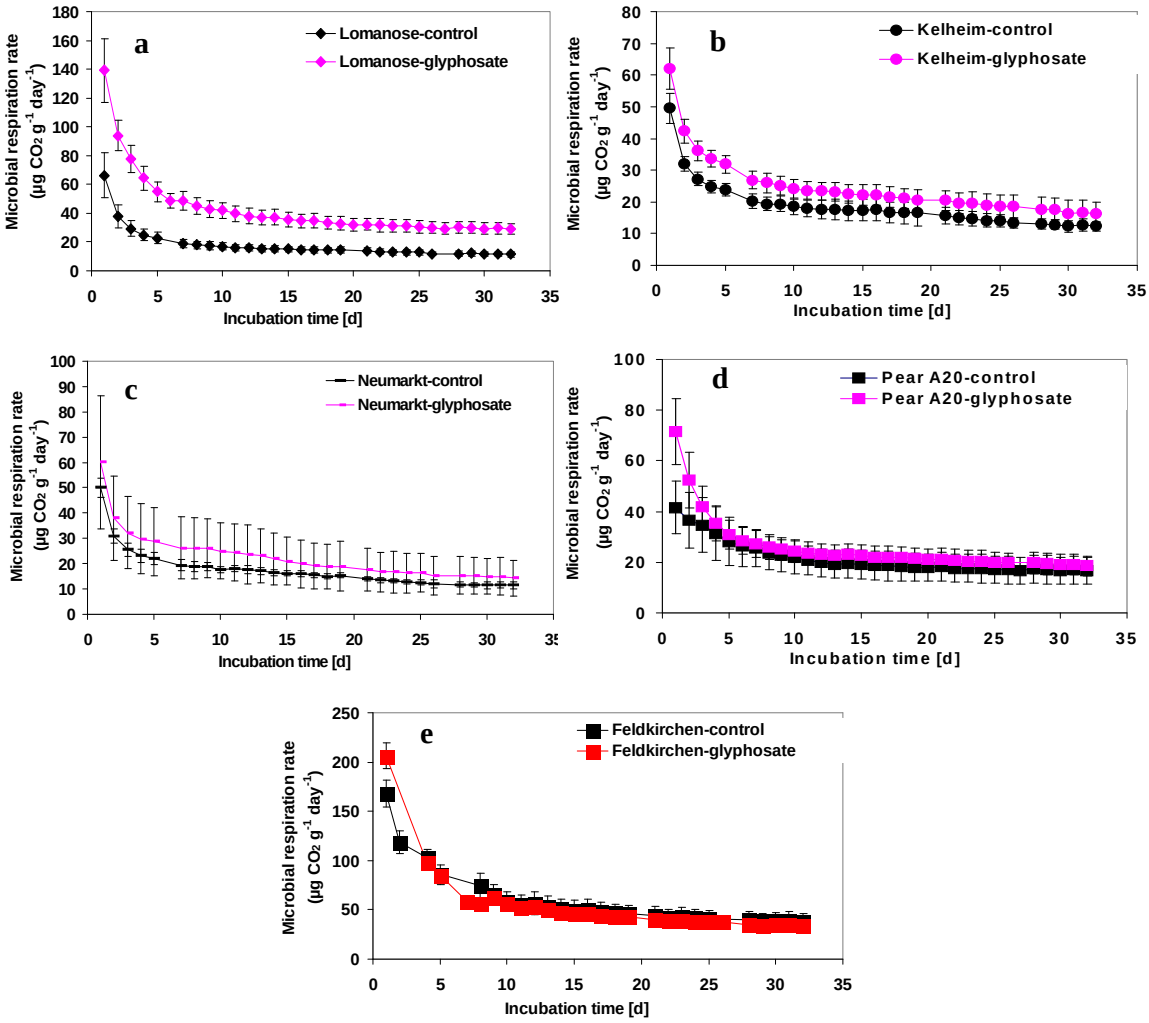


Figure 5.39. Development of microbial respiration rate in soils with and without 10 µg glyphosate g^{-1} soil in course of 32 day incubation: examples for stimulating effect of glyphosate on soil respiration, Lomanose (a), Kelheim (b), Neumarkt (c), Pear A20 (d), and Feldkirchen (e) (bars indicate standard deviation)

The depressing effect of glyphosate on soil respiration is depicted on Figure 5.40. Out of 21 soils examined, only 3 soils (Ada A02, Skrinjar and Hohenwart) showed a depressing effect of glyphosate on soil respiration. Out of these 3 soils, only Ada A02 soil showed a significant difference between the glyphosate treated and the non treated variant.

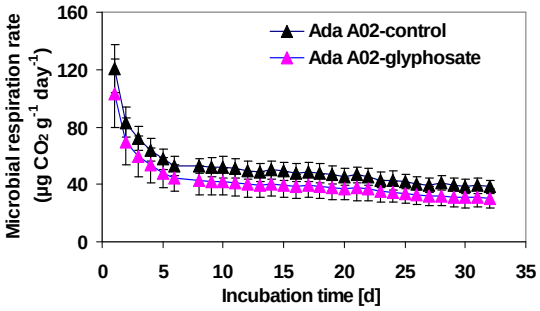


Figure 5.40. Development of microbial respiration rate in soil Ada A02 with and without 10 µg glyphosate g^{-1} soil in course of 32 day incubation: example for depressing effect of glyphosate on soil respiration (bars indicate standard deviation)

Fifteen out of 21 soils showed no effect of glyphosate on soil respiration. There was no significant difference between the respiration rates in the glyphosate treated variants and the non treated variants of the following soils: Apace-njiva, Berta A02, Brezje, Dunjar A06, Grace A13, Hanna A15, Joy A19, Konjise, Lamanose, Scheyern-lysi, Zepovci, Skrinjar, Hohenwart, Lea A18 and Zepovci (Plitv.). Figure 5.41 presents only 2 soils as an example for no effect of glyphosate on soil respiration.

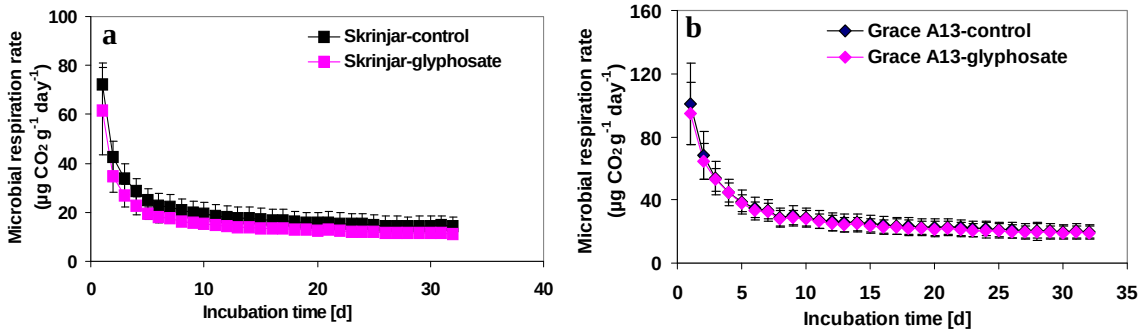


Figure 5.41. Development of microbial respiration rate in soils with and without 10 µg glyphosate g^{-1} soil in course of 32 day incubation: examples for no effect of glyphosate on soil respiration, Skrinjar (a) and Grace A13 (b) (bars indicate standard deviation)

6. Discussion

The general objective of the study was to investigate the parameters and processes that govern biomineralization and bioavailability of glyphosate in 21 different agricultural soils. The main working hypothesis of the study was that bioavailability is a factor most strongly governing biomineralization of glyphosate in soil. Two approaches were used to measure the bioavailability of glyphosate by non-biological techniques: the classical OECD approach and an *in situ* pore water approach. It should be tested which one of the two approaches is the best one to determine *in situ* bioavailability whereby glyphosate mineralization in soils served as the reference for real bioavailability. For further elucidation of the processes behind bioavailability and mineralization detailed studies were conducted to characterize the mineralization dynamics and glyphosate uptake dynamics by microbial cells.

6.1. Degradation and biomineralization of glyphosate in different agricultural soils

The biomineralization of glyphosate in 21 agricultural soils was highly varied (between 7.64 and 68.70 % of the applied ^{14}C-glyphosate) (Figure 5.1). Similar results were found by Gimsing et al. (2004a). The authors of the study found that the mineralization of glyphosate after 92 days varied approximately from 3 to 45 % of applied glyphosate among 8 different soils. High mineralization of glyphosate in soils was reported by Nomura and Hilton (1977); Smith and Aubin (1993); Cheah et al. (1998); Wiren-Lehr et al. (1997); Andrea et al. (2003); Klier (2007); Bonfleur et al. (2010). The big difference in biomineralization of glyphosate among 21 soils indicates that agricultural soils have different abilities to degrade glyphosate. This can be ascribed to differences in the soil characteristics (chemical, physical and microbial properties). For most of the 21 soils a rapid mineralization of glyphosate was observed during the first 4 days without a lag phase, but mineralization rates subsequently decreased over time, as also found in other earlier studies (Moshier and Penner, 1978; von Wiren-Lehr et al., 1997; Eberbach, 1998; Gimsing et al., 2004a). This could be attributed to processes of sorption and desorption resulting in the reduction of glyphosate bioavailability to soil microorganisms over time (Gimsing et al., 2004a).

The mineralization rate of glyphosate in the two soils, Konjise and Joy A19, increased slightly to a maximum (5.0 % and 4.3 % of the initial ^{14}C-glyphosate per day, respectively) after 4 days (Figure 5.2b). Thereafter there was a continuous decrease until end of the experiments. The rest of the soils had only a continuous decrease of mineralization rate until end of the experiments. This seems that bioavailability of glyphosate in these two soils was

not a limiting factor to glyphosate mineralization during the first 4 days of the incubation time. This finding seemed to be contrary to other previous studies and the result from the other soils in this study which have shown that the degradation rate of glyphosate rapidly declined initially (Rueppel et al., 1977; Moshier and Penner, 1978; Carlisle and Trevors, 1988; Hansen et al., 2004; Sorensen et al., 2006).

6.1.1. Soil properties governing mineralization of glyphosate

Several microbial and physico-chemical soil parameters have been related to degradation of glyphosate in soils such as soil microbial biomass soil respiration rate, the soil pH, soil organic carbon content, clay content, phosphorous content, Al/Fe-oxides, CEC and Cu^{2+} content. Although glyphosate sorption and desorption in agricultural soils have been intensively investigated, information concerning specially the role of soil properties on mineralization of glyphosate is quite limited.

Like other previous studies, the univariate correlations between mineralization glyphosate and soil parameters were also calculated in this study. Results show that the difference in cumulative mineralization among soils was strongly and significantly governed by exchangeable $[H^+]$. Besides, the cumulative mineralization of glyphosate in soils was significantly correlated with soil pH, oxalate extractable Al and bacterial cell numbers at the end of the experiment. This indicates that the cumulative mineralization of glyphosate is governed by both microbiological and chemical properties of the soils (Albers et al., 2009; Kim et al., 2011).

The fact that there was low mineralization of glyphosate in soils which have high amount of exchangeable H^+ illustrates that the exchangeable H^+ interfered with the mineralization process in soils. This could be explained by the fact that a binding between the carboxylic (or phosphonic acid) groups of glyphosate and exchangeable H^+ is formed (Shoval and Yariv, 1979). Therefore, the bioavailability of glyphosate is reduced in soil with high exchangeable H^+. This is supported by the fact that there is a significantly negative correlation between dissolved glyphosate (OECD) and exchangeable H^+ in soils (Figure 5.17a). Besides, it is also possible that glyphosate can exchange with H^+ for sorption on soil or clay minerals. If an exchange between glyphosate and H^+ in soils happens, more exchangeable H^+ could be released to soil solution, then it partly influenced on the microbial activity as result of reducing the soil pH. However, the data for soil respiration does not show any relation with

exchangeable H^+. This also can be assumed that the glyphosate degrading microorganisms were more sensitive to low soil pH than the total heterotrophic soil micro-flora. Unfortunately, there is no information in literature about this fact. Therefore, it is necessary to clarify if exchangeable H^+ makes glyphosate un-bioavailable to mineralization or causes unfavourable conditions for glyphosate degrading microbes in future work.

A positive correlation between cumulative mineralization and soil pH was found. This finding is not consistent with the findings of Kools et al. (2005) who found a negative correlation between glyphosate degradation and soil pH. Soil pH affects the adsorption of available glyphosate fraction, which is stronger in acidic soils (Eberbach, 1998; Kools et al., 2005). The effect of soil pH on adsorption of glyphosate in soils can be explained by the fact that when pH increases, glyphosate molecule can form more negative charges and in the same condition, clay minerals, aluminum and iron oxides increase negative charges as well. Therefore, glyphosate can be repelled by negative charge surfaces and accordingly the adsorption of glyphosate in soil decreases (McConnel and Hossner, 1985; Morrillo et al., 1997; Rafiei Keshteli et al., 2011). Additionally, soil pH itself might directly affect the availability of organic and inorganic nutrients, thus affecting the size and diversity of microbial community (fungi, bacteria) in the soil conditions of low soil pH (Sorensen et al., 2006). Therefore, the influence of the soil pH on the bacterial community responsible for glyphosate degradation in soil can be of importance (Kools et al., 2005).

Bacterial cell counts at the end of the experiments were found to be positively correlated with cumulative mineralization of glyphosate. This study result was in contrast with previous studies of Gimsing et al. (2004a) and Castillo et al. (2010) which have shown that no correlation between bacterial cell counts and mineralization of glyphosate in soils was found. However, they found a positive correlation between the mineralization of glyphosate and numbers of *Pseudomonas* sp.. Moreover, Castillo et al. (2010) also found that applying of glyphosate into soil stimulated the increase of population of *Pseudomonas sp*.. Therefore, it can be assumed that the bacterial cell numbers at the end of the experiment seemed to be the degrading microorganisms for glyphosate in soils and it was likely that microbes capable of degrading glyphosate aerobically exist in soils.

In this study, the effect of oxalate extractable Al^{3+} on mineralization of glyphosate was not as strong as exchangeable H^+ although there was a significant correlation between

mineralization of glyphosate and oxalate extractable Al^{3+}. Therefore, adsorption of glyphosate on Al-oxides or the complexation of glyphosate with oxalate extractable Al^{3+} led to reduced glyphosate mineralization (Sheals et al., 2002; Melissa et al., 2012).

The strong correlations of cumulative mineralization of glyphosate with exchangeable H^+, soil pH, oxalate extractable Al^{3+}, and bacterial cell counts at the end of the experiments (Figure 5.3) show that these parameters can primarily be used as indicators of glyphosate degradation. It is not surprising when it was found in this study that exchangeable H^+ and soil pH individually govern not only the mineralization of glyphosate but also the adsorption (Figure 5.17) and desorption (Figure 5.21) of glyphosate in soils. This confirms that the degradation of glyphosate is primarily regulated by adsorption and desorption processes in soils.

Most studies for glyphosate from the literature have been focused very much on adsorption of glyphosate in relation to soil parameters with very little in relation to soil varieties. Only univariate regression has been used to correlate mineralization or adsorption of glyphosate with each single soil parameter in previous studies (Hancem 1976; Gimsing et al., 2004b; Mamy and Barriuso, 2005; Rafiei Keshteli et al., 2011). Univariate regression does not have any combination of soil parameters. The soil parameters do not work separately, but they interact with each other to have a complexity in soil matrix. Therefore, to be able to understand which soil parameters interact with one another to regulate the mineralization of glyphosate in soils, a multiple regression analysis was conducted. The result of multiple regressions is that exchangeable $[H^+]$, $[Ca^{2+}]$ and $[K_2O]$ are the most important factors collectively contributing to the cumulative mineralization of glyphosate in soils (model A). In this multiple regression, exchangeable H^+ has a negative correlation with mineralization of glyphosate, whereas exchangeable Ca^{2+} and K_2O have a positive correlation with cumulative mineralization of glyphosate. Once again, this result indicates that exchangeable H^+ is an important factor which reduces the bioavailability of glyphosate in soils, and as a consequence the mineralization of glyphosate is reduced. Regarding Ca^{2+} and K_2O, cumulative mineralization was found to be positively correlated with exchangeable Ca^{2+} and K_2O, respectively. This is not consistent with the result study of Melissa et al. (2012). In their study they showed that glyphosate formed a metallic complex with Ca^{2+} in soil. But there is no information regarding the effect of K_2O on mineralization or adsorption of glyphosate in soils. Therefore, it is proposed in this study that a complex between glyphosate with

exchangeable Ca^{2+}/K_2O will not reduce the bioavailability and mineralization of glyphosate. In the contrary, Ca^{2+}-glyphosate complexes may be transported more efficiently across microbial cell walls than sole glyphosate compound as it has already been argued for Cu^{2+} complexes in literature (Kools et al., 2005). However, these mechanisms have not been documented and should be clarified.

6.1.2. Soil properties governing NaOH extractable residues

The NaOH extractable residues are relatively high and variable. Between 23 and 91 % of initial glyphosate after 32 days incubation are extracted with NaOH 0.1M (Table 5.1). This indicates that the extractable residues of glyphosate in soils can be effectively extracted with NaOH. A strong and negative correlation between cumulative mineralization within 32 days and the NaOH extractable residues was found. This shows that NaOH extractable residues were non-available for microorganisms to be degraded. This result is not in accordance with the study of Gimsing et al. (2004a) who showed that glyphosate adsorbed to iron or aluminum oxides in the soils can be desorbed and subsequently mineralized. However, according to Stenrod et al. (2005) NaOH extraction could extract both bioavailable and non-bioavailable glyphosate in soils. Besides, the fact that ^{14}C-glyphosate is the major component in the NaOH extract (Table 5.2) as compared to AMPA and unknown metabolites and that a strong, significant and positive correlation between NaOH extractable residues and the amount of ^{14}C-glyphosate in NaOH extract is found indicates that in soils with low mineralization glyphosate is present in a high amount and that this glyphosate could not be degraded / mineralized because it was adsorbed to Al- or Fe-oxides. Gimsing et al. (2004a) also found glyphosate as a major component in the NaOH extract. This might be a hint that adsorbed glyphosate by Al/Fe-oxides is slowly released to soil solution and as long as glyphosate is degraded to degradation products in soil solution by microorganism, the degradation products are quickly mineralized to CO_2. However, neither the correlation between the mineralization of glyphosate and NaOH extractable residues nor the correlation between NaOH extractable residues and glyphosate in NaOH extract have been given in the literature.

The NaOH extractable residue is strongly correlated not only with Al-oxides, but also with other soil parameters, e.g. exchangeable H^+, soil pH, bacterial cell counts at the end of the experiment. This indicates that NaOH extractable residues are influenced not only by chemical soil properties, but also by microbiological factors (Figure 5.4). NaOH extractable fraction can use to be interpreted not only as the adsorbed amount of glyphosate to iron and

aluminum oxides, but also as the adsorbed amount of glyphosate that is exchangeable with H^+. These soil parameters are identified as individually regulating parameters for the adsorption capacity of glyphosate in soils. Bacterial cell counts at the end of the experiments also influence the NaOH extractable residues. This might be that the bacterial cell numbers which are cultivable in agar plate are glyphosate degrading microorganisms in soils. Therefore, at the end of the experiment soils with higher bacterial cell numbers (also higher mineralization of glyphosate) resulted in less amounts of NaOH extractable residues. However, no information from the literature has shown that bacterial cell numbers has a positive correlation with either mineralization of glyphosate or NaOH extractable residues.

Multiple regression analysis was conducted to investigate the interacting functions of the different soil parameters on NaOH extractable residues. The result shows that the soil parameters which collectively control most of the extractable residues are exchangeable H^+ and CEC (model B). This indicates that NaOH extractable residues can partly be interpreted as an adsorbed amount of glyphosate to Al-oxides, but it seems to be mainly interpreted for the amount of glyphosate adsorbed on exchangeable H^+. Actually H^+ also belongs to CEC, but why does NaOH extractable residues have positive correlation with H^+, whereas it has a negative correlation with CEC including other cations: Ca^{2+}, Mg^{2+}, K^+ and Na^+? It is most likely contributed by Ca^{2+} since the Ca^{2+} is the most predominant cation in total CEC. Additionally, according to a multiple regression analysis for mineralization, Ca^{2+} which is one of the most important parameters has positive correlation with mineralization of glyphosate in soil. Therefore, soils which have high Ca^{2+} (CEC) result in low amount of NaOH extractable residues and respectively in low amount of glyphosate residues since glyphosate is the major component of NaOH extractable residues. This means that glyphosate degradation and mineralization is increased at high Ca^{2+} content in soils and this also means that the above mentioned hypothesis that Ca^{2+}-glyphosate complexes are transported more efficiently across microbial cell walls than sole glyphosate compound is strengthened by the negative correlation between Ca^{2+} and NaOH extractable residues.

6.1.3. Soil properties governing non-extractable residues

Non extractable residues have a strong correlation with soil pH, cumulative mineralization of glyphosate and exchangeable H^+. This indicates that both soil chemical and microbial properties influence the formation of NER. Soil pH seems to be the most important factor governing the bound residues of glyphosate in soils. With increasing soil pH the

formation of bound residues increases. This is accordance with the study of Abou-Assaf et al. (1987) who also found that the soil bound residues of the organophosphate pesticide isophenphos increased in alkaline soil compared with neutral and acidic soils. Although the formation of soil bound residues of glyphosate has been reported (Andrea et al., 2003; Mamy et al., 2005; Weaver et al., 2007; Zablotowicz et al., 2009) it was not related to soil pH.

The high mineralization of glyphosate in soils coincided with non extractable residues at the end of the experiment. This could be an argument that the bound residues in the present study are partly formed by an incorporation of ^{14}C-glyphosate into microbial biomass (Charnay et al., 2004), and that the microorganisms were able to utilize glyphosate for growth-related metabolism (Lancaster et al., 2010). Moreover, glyphosate and degradation products can form bound residues by themselves.

The fact that the formation of NER decreases when exchangeable H^+ increases (Figure 5.6b) indicates that the exchangeable H^+ did not trigger the formation of bound residues in soils. This could be due to the direct negative influence of exchangeable H^+ on soil microorganisms, particularly, glyphosate degrading microorganisms when glyphosate is applied. This is supported by the fact that both cumulative mineralization and NER have significantly negative correlation with exchangeable H^+.

Multiple regression analysis was conducted to investigate the interacting functions of the different soil parameters on NER. The result showed that soil pH, water content at -15 kPa and oxalate extractable Al^{3+} are soil parameters governing most the formation of bound residues of glyphosate (model C). This means that the bound residues of glyphosate increases in soils which have high soil pH, high water content at -15 kPa, and low oxalate extractable Al^{3+}. Although the microbial factors were not included in the equation which shows important factors for bound residues, it does not mean that the role of microorganisms was small. This could be due to the indirect influence of microorganisms on NER of glyphosate. This is supported by an appearance of pH and water content in the equation. These 2 soil parameters are also favorable factors for the activity of microorganisms in soils. Therefore, it could be concluded, that the microbial activity is a main process to form bound residues of glyphosate in soils. Oxalate extractable Al^{3+} additionally contributes to soil pH and water content at -15 kPa to the bound residues of glyphosate in soil. This could be an argument that glyphosate that is adsorbed by Al or Fe-oxides is not bioavailable to be degraded by soil microbes

Therefore, the bound residues of glyphosate was formed during the biodegradation experiments only as the result from microbial activity.

6.1.4. ^{14}C-glyphosate residues in soil pore solution

The results show that the amount of ^{14}C-glyphosate residues in soil pore solution is very low, lower than 0.4 % of the initial ^{14}C-glyphosate. In contrast, the mineralization rate is much higher than dissolved glyphosate in soil pore solution. This result is consistent with the study by Stenrod et al. (2005) who have also shown that ^{14}C-glyphosate in soil pore solution was also very low, <0.2% of initial applied ^{14}C at day 0 and <0.1% at later samplings. Therefore, the result indicates that soil water extraction via centrifugation procedure did not constitute the entire bioavailable fraction of glyphosate in soils. As will be shown later, the uptake of glyphosate by soil bacteria accounts for this discrepancy.

6.1.5. Bacterial cell counts before and after the biodegradation experiments

The CFU numbers after the biodegradation experiments changed in some soils whereas in some other soils the CFU numbers did not change. This shows that the effect of experimental conditions and glyphosate on culturable microorganisms differs from soil to soil. However, the increase or decrease in CFU numbers at the end of the experiment as compared to CFU numbers at the beginning of the experiment (no glyphosate application) was most likely effected by glyphosate because the experiments were conducted under the conditions which are optimal for the microbial activity (Ilstedt et al., 2000; Schroll et al., 2006). The fact that CFU numbers increased in soils: Ada A02, Apace-njiva, Berta A02, Brezje, Dunjar A06, Konjise, Skrinjar and Zepovci (Plitv.) at the end of the biodegradation experiments indicates that glyphosate might serve bacteria as nutrient source for their growth. However, the increase of CFU numbers in these soils did not coincide with the soil respiration. In other words, an increase of CFU numbers at the end of the experiment in these soils did not result in an increase of soil respiration. This indicates the culturable bacteria which were stimulated by addition of glyphosate contributed a small proportion of a whole microbial community in these soils. This is in accordance with previous studies by Sprankle et al. (1975a); Carlisle and Trevors, 1988; Dick and Quinn, 1995; Partoazar et al. (2011) who found that glyphosate can be present in soil as C, N and P sources for bacterial community. The function of glyphosate as substrates for the direct metabolism leading to microbial biomass and activity has been also shown by Haney et al. (2000); Wardle and Parkinson (1990). A strong decrease in bacterial cell numbers was found in soils: Grace A13,

Hohenwart, Lomanose, Neumarkt, Pear A20 and Schyern-lysi. This could be attributed to the toxicity effect of glyphosate on bacteria. However, no reduction of soil respiration was found in these soils (except for soil Hohenwart). The result illustrates that the culturable bacteria which were depressed by adding glyphosate contributed a small proportion of a whole microbial community in these soils. A decrease in bacterial number by applying glyphosate in soil was also found by Mekwatanakarn and Sivasithamparam, 1987 and Araujo et al. (2003).

6.2. Soil properties governing dissolved glyphosate (OECD)

Earlier studies showed that glyphosate was rapidly and strongly sorbed to soil matrix. Sorption of glyphosate in soils also depends very much on soil parameters such as soil pH, clay content, soil organic carbon content, phosphorous content, Al/Fe-oxides, Cu^{2+} content, and CEC (Baylis, 2000; Veiga et al., 2001; Andrea et al., 2003; Kogan et al., 2003; Hansen et al., 2004; Autio et al., 2004; Mamy et al., 2005; Borggaard and Gimsing, 2008; Rafiei Keshteli et al., 2011).

The dissolution of glyphosate in soil determined by OECD guideline 106 had a positive correlation with soil pH whereas dissolution of glyphosate (OECD was found to be negatively correlated with exchangeable H^+ and clay content in soils (Figure 5.17). This indicates that exchangeable H^+, soil pH and clay content played an important role in regulating dissolution of glyphosate in soils. The dissolution of glyphosate in soils increased when soil pH increased. Conversely, dissolution of glyphosate in soils decreased when exchangeable H^+ and clay content in soils increased. This is consistent with the findings of Gimsing et al. (2004b) and Mamy and Barriuso (2005) who found that the dissolution of glyphosate in soil was positively correlated with soil pH. The effect of soil pH on adsorption of glyphosate can be explained by the soil pH depending on charges of glyphosate molecules and clay minerals. In a high soil pH condition, both glyhosate molecules and clay minerals, e.g. Al/Fe-oxides produce the same negative charges, therefore, the sorption of glyphosate on soils in this case reduces. A significant and positive correlation between mineralization of glyphosate at day 1 and dissolved glyphosate (OECD) (Figure 5.15) shows that the adsorption and dissolution of glyphosate directly regulated the degradation of glyphosate in soils. This is supported by the fact that both glyphosate adsorption and cumulative mineralization had a significantly negative correlation with exchangeable H^+, but had positive correlation with soil pH. Therefore, it is most likely that exchangeable H^+ and soil pH are important soil parameters controlling adsorption and subsequently regulating the degradation of glyphosate in soils.

Although many studies concerning adsorption of glyphosate on soils have been documented (Hance, 1976; Glass, 1987; Calvet, 1989; Piccolo et al., 1994; Kogan et al., 2003; Autio et al., 2004; Zhou et al., 2004; Yu and Zhou, 2005; Cruz et al., 2007; Jensen et al., 2009; Rafiei Keshteli et al., 2011), almost no correlation was calculated in relation between soil parameters and adsorption of glyphosate since in their studies only a small number of f soil samples was selected.

Multiple regression analysis was conducted to investigate the interacting functions of the different soil parameters on glyphosate adsorption in soils. This means that a combination of parameters which in themselves don't significantly correlate with glyphosate adsorption can explain the variation (Gimsing et al., 2004b). The result shows that glyphosate adsorption could be well described by a model D which is combined by 3 soil parameters (soil pH, organic carbon content and silt content). The importance of soil pH for adsorption of glyphosate has previously been shown (McConnell and Hossner 1985; Gimsing et al., 2004b; Mamy and Barriuso, 2005; Cruz et al., 2007; Borggaard and Gimsing, 2008; Jensen et al., 2009). The role of organic carbon content in adsorption of glyphosate has also been documented (Yu and Zhou., 2005; Accinelli et al., 2005; Zablotowicz et al., 2009; Albers et al., 2009; Rafiei Keshteli et al., 2011). However, the importance of silt content in adsorption of glyphosate has been not documented yet. From the result of multiple regression analysis for mineralization hypothesises are arisen as follows: i) significant univariate correlation between dissolved glyphosate and the parameters exchangeable H^+, K_2O, and Ca^{2+} is found and ii) multivariate regression analysis should also include exchangeable H^+, K_2O, and Ca^{2+} as the main governing parameters for adsorption and iii) correlation between sorption and mineralization is found. However, the parameters that govern adsorption (OECD) are not the same that were identified to govern mineralization. The parameters which are found to govern mineralization are parameters which have an effect on the sorption behavior of chemicals. Therefore, the parameters which govern adsorption should be the same as the parameters which govern mineralization. But this is not the case. Possible reason is that the artificial conditions of the OECD approach do not produce realistic results and therefore identical governing parameters for mineralization and adsorption (OECD) were not found.

6.3. Soil properties governing dissolved glyphosate (PW)

A comparison of two methods (OECD and PW) in determining dissolved glyphosate in soils shows that with OECD approach more glyphosate was found dissolved. When using the

PW approach, dissolved glyphosate in soil pore water was very low (Figure 5.13 and 5.14). This might be logical and this result agrees with earlier study of Folberth et al. (2009a) who also showed that the amount of dissolved herbicide isoproturon was higher when using OECD approach as compared to PW method. This could be due to artificial experimental conditions since using OECD approach with a high amount of aqueous phase and vigorous shaking, soil structures, especially soil aggregates can be changed (Wauchope et al., 2002) while the experimental conditions in PW approach are closer to realistic conditions like they are present in natural soils. Furthermore, the PW approach avoids as much as possible the artifacts which can result in a wrong interpretation of the facts happening in natural soils. This is the reason why *in situ* sorption experiments by PW approach were additionally conducted. The conditions of these experiments are much more realistic and they should therefore produce more realistic results. Moreover, the mineralization experiments were conducted under the same conditions as the *in situ* sorption experiments. But it was shown that no reasonable correlation is found when taking 21 soils into account for univariate correlation between dissolved glyphosate and soil parameters in the PW approach. Furthermore, no reasonable correlation between dissolved glyphosate (PW) and mineralization at day 1 is found and the mineralized amount of glyphosate at day 1 is much higher than the dissolved amount of glyphosate determined with PW. Now the question arose, what could be the reason for that lacking correlation between mineralization and sorption behaviour of glyphosate? One reason could perhaps be the high NaN_3 concentration which has been taken from the literature. Therefore, additional *in situ* experiments with and without NaN_3 addition to soil were conducted to check effect of NaN_3 on the quality of soil pore water. And it turned out that the pore water of the soils with NaN3 addition showed a reddish colour and lower soil pH while the pore water without NaN3 addition was not coloured (Table 5.5). This does not agree with results of studies by Parochetti and Warren (1970) and Wolf et al. (1989) which have shown that the soil pH significantly increased in soils treated with 200 µg NaN_3 g^{-1} soils. This shows clearly that in the presence of NaN_3, Fe (III) was dissolved in the soil solution by forming a complex with N_3^-. It is a freely soluble complex in aqueous phase with a dark red color (Betterton, 2003 and Burgess and Twigg, 2006). This results in a decrease in the adsorption of glyphosate on Al/Fe-oxides sites because Al/Fe-oxides in soils are clocked by NaN_3. Thus, sorption behaviour of glyphosate was strongly influenced by the high NaN_3 concentrations and therefore reasonable correlations between mineralization and soil parameters and sorption and soil parameters could not found. Because of the effect of NaN_3 on iron the sorption results are falsified and it is not astonishing that there was no correlation between

mineralization and dissolved glyphosate. In conclusion, there are many artificial results in literature because of the application of high NaN_3 concentrations. These results must be re-evaluated.

A comparison concerning dissolution of pesticides in soil pore water within PW extraction between isoproturon [Folberth et al. (2009a)] and glyphosate in this study shows that the dissolution of isoproturon was much higher than that of glyphosate in the same soil. This indicates glyphosate is really strongly absorbed in soil matrix as compared to isoproturon. Moreover, there is a positive correlation between dissolved isoproturon in soil pore water and mineralization, but in this study no reasonable correlation between dissolved glyphosate in soil pore water and mineralization. Interestingly, in case of glyphosate, the mineralization in all studied soils at day 1 is much higher than the dissolved glyphosate (PW). This is partly attributed to an uptake process of glyphosate by microorganisms in soil and, the large amount of glyphosate taken up by microorganisms shortly after application can be mineralized over a long term period. As will be shown later, the uptake of glyphosate by soil bacteria accounts for this discrepancy.

6.4. Soil properties governing desorbed glyphosate (OECD)

Same pattern as dissolved glyphosate in soil, desorbed glyphosate using PW approach was much lower than that using OECD approach. However, the desorption of glyphosate in soil using PW approach was performed only in 3 soils, whereas 21 soil samples were determined for desorption of glyphosate with OECD method. Therefore, in this part desorbed glyphosate in OECD approach is only discussed. The cumulative desorbed glyphosate within 6 days also means for the cumulative dissolved glyphosate in soil pore water. The fact that there is a positive correlation between the cumulative mineralization of glyphosate within relatively 6 days and cumulative desorption of glyphosate within relatively 6 days (Figure 5.19) indicates that the desorptipon of glyphosate is an important process regulating the mineralization in soils. The fact that cumulative mineralization, dissolution and desorption of glyphosate were significantly and strongly correlated with exchangeable H^+ and soil pH argues that cumulative mineralization, adsorption and desorption of glyphosate in soils are pH-dependent. The adsorption and desorption processes in soils directly govern the mineralization of glyphosate. This is consistent with the findings of Piccolo et al. (1994) and Al-Rajab et al. (2008) who also found that the adsorption and desorption of glyphosate were

pH-dependent. The information regarding the relation between mineralization and desorption in soils from the literature is still missing.

Multiple regression analysis was conducted to investigate the interacting functions of the different soil parameters on glyphosate desorption in soils. The result shows that glyphosate desorption could be well described by a model E which is combined by 3 soil parameters (exchangeable H^+; soil pH, and Mg^{2+}). It means that desorption of glyphosate in soils has positive correlation with soil pH, but has negative correlation with exchangeable H^+ and Mg^{2+}, respectively. The importance of soil pH for desorption of glyphosate has previously been shown (Piccolo et al., 1994; Sorensen et al., 2006; Al-Rajab et al., 2008). However, the importance of exchangeable H^+ and Mg^{2+} for desorption of glyphosate has not been documented yet. This result reveals that glyphosate that is adsorbed by exchangeable H^+ and Mg^{2+} is not easy to be desorbed to soil solution. In conclusion, exchangeable H^+, soil pH and Mg^{2+} were soil parameters regulating collectively the desorption of glyphosate in a combination of soil parameters.

6.5. Quantification of glyphosate in soil pore solution shortly after application

The cumulative mineralization of glyphosate in soil Feldkirchen was approximately 30 % of the initial applied ^{14}C after 3 days (Figure 5.22a). This is in accordance with the biodegradation experiment in soil Feldkirchen which was conducted earlier in this study. This result shows that the mineralization of glyphosate in soil Feldkirchen is very high. The biodegradation experiment with NaN_3 application showed that only a small amount of glyphosate was mineralized after 3 days (0.04 % of the initially applied ^{14}C; Figure 5.23). This attests to the effectiveness of the sterile condition by using 6,500 µg NaN_3 g^{-1} soil. The fact that CFU numbers in soil Feldkirchen did not increase after glyphosate application (Figure 5.24) indicates that glyphosate did not make any change of the CFU numbers of this soil. It could be because soil bacteria could not use glyphosate as carbon sources for their growth. Glyphosate in soil pore water was gradually reduced during the biodegradation experiment without NaN_3 (Figure 5.25). This is attributed to biodegradation of dissolved glyphosate in soil pore water by soil microorganisms. The fact that the real *in situ* bioavailability of glyphosate under biotic conditions was higher than that under abiotic conditions determined by PW approach could be explained by a great contribution of soil bacteria. Bacteria compete with soils for the uptake of glyphosate. Therefore, in abiotic conditions without microbial activity dissolved glyphosate in soil pore water was lower

because glyphosate was adsorbed more into soil matrix. Conversely, in the case of biotic conditions with microbial activity, 2 processes took place after glyphosate was applied: (1) glyphosate is adsorbed into soil and (2) glyphosate is taken up by bacteria. Actually, glyphosate which is taken up by bacteria is bioavailable for mineralization later. Because glyphosate is taken up by soil microbes, our method of using centrifugation procedure to extract dissolved glyphosate in soil pore water did not constitute the entire bioavailable fraction of glyphosate. Therefore, the real *in situ* bioavailability of glyphosate in soils is higher than the bioavailability of glyphosate determined by PW approach.

6.6. Glyphosate biomineralization capacity of the soil microbial community in nutrient solution

In nutrient solution only glyphosate was added as carbon and phosphorous sources. All treatments which received differently initial applied CFU numbers showed maximum CFU development at day 3. This indicates that the bacterial community survived in a short period of time. A short period for bacterial growth in CFUs after 3 days was also shown by Kolawole and Akinsoji (2011). They demonstrated that glyphosate in nutrient solution containing 50 µg glyphosate mL^{-1} was completely degraded to zero after 3-4 days incubation which coincided with a reduction of CFU numbers. The fact that a higher biomineralization of glyphosate was observed in the treatment initially incubated with high CFU numbers (Figure 5.28) confirms that biomineralization of glyphosate in nutrient solution which has no sorption sites for glyphosate depends very much on bacterial numbers and that glyphosate can be degraded by a broad range of bacteria (Dick and Quim, 1995). Moreover, at day 1, the mineralization rate of glyphosate per CFU per day was lowest in the treatment initially incubated with high CFU numbers as compared to two others (Figure 5.30). This shows that only a few bacteria of a whole community could use glyphosate at day 1 as nutrient sources, whereas most bacteria might have used dissolved organic carbon extracted from soils during the microbial extraction step for their growth. After day 1, this carbon source seemed to be used up. Therefore, all bacteria used glyphosate as nutrient sources. This coincided with the higher mineralization rate of glyphosate per CFU per day in the treatment initially incubated with high CFU numbers.

6.7. A comparison of glyphosate biodegradation capacity in soil and nutrient solution

The purpose of this comparison is to test whether the glyphosate mineralization is enhanced in nutrient solution which has no adsorption sites for glyphosate. The result shows

that at day 1 the mineralization rate per CFU per day of glyphosate in the nutrient solution treatment initially incubated with $13.5x10^6$ CFUs in 50 mL medium was significantly higher than that in the soil treatment initially with $13.5x10^6$ CFUs 30 g^{-1} soil. This can assume that the free adsorption sites for glyphosate may affect the mineralization of glyphosate under nutrient solution conditions. Such condition may not be available under soil condition. Under soil conditions, soil bacteria have to compete with soils for the uptake and mineralization of glyphosate. However, in nutrient solution media, glyphosate is available for bacteria to take up and mineralize. At day 3, the mineralization rate of glyphosate per CFU per day in the soil treatment was significantly higher than that in all the nutrient solution treatments. This could be because of the high increase of the bacterial cells in the nutrient solution from day 1 to day 3 as compared to bacterial cell numbers in soil (Figure 5.34). The proportion of non-glyphosate degrading bacteria in a bacterial community growing during the first 3 days in nutrient solution was larger than that of glyphosate degrading bacteria. Therefore, when calculation for mineralization rate per CFU per day, the mineralization rates per CFU per day were lower in nutrient solution treatments than in the soil treatment. The fact that the growth of CFU numbers was not observed in soil media, but in the nutrient solution treatments can be explained as follows: (1) bacteria in soils did not use glyphosate as carbon sources for their growth; (2) the carbon sources for the growth of bacteria in nutrient solution could come from glyphosate or from other available carbons e.g. dissolved organic carbon, which have origin from the soil during the microbial extraction step. However, the difference between glyphosate biodegradation in soil and nutrient solution treatments is just small. This is probable that in soil media, water content was lesser than in nutrient solution. The diffuse distance between glyphosate and microbes was shorter in soil than in the nutrient solution. Thereby, although the adsorption sites for glyphosate was absent in nutrient solution, the mineralization rate per CFU per day of glyphosate in nutrient solution was not much higher than in the soil media. However, there exists no information concerning diffusion of glyphosate in soils and in nutrient solution from the literature.

6.8. Dynamics of glyphosate biomineralization and glyphosate uptake by soil microorganisms in nutrient solution

The uptake of glyphosate by bacteria increased over time (Table 5.8). This can be explained by an increase of CFU numbers during the first 3 days from the nutrient solution experiment which was conducted earlier in this study (Figure 5.27). Therefore, the result indicates the amount of glyphosate taken up by soil microorganisms depends on the bacterial

cell numbers. After 0.17 day incubation in the nutrient solution phase I, a large amount of glyphosate was taken up by bacteria (2.4 % of the initial glyphosate), but the glyphosate taken was mineralized in a long time (Table 5.8). In the nutrient solution phase II, the mineralization of glyphosate that was taken up by microbial cells was very low and lasted for some days (6-10 days). This could be explained by the fact that the live bacteria at that time used death cells as carbon sources for their growth. Therefore, the mineralization of glyphosate started because degradation of glyphosate was co-metabolical with bacteria using glyphosate as phosphorous sources (Talbot et al., 1984; Kishore and Jakob, 1987; Dick and Quinn, 1995). In conclusion, the soil bacteria have capacity to take up glyphosate and glyphosate taken up by soil bacteria is mineralized over a long term period. The uptake of glyphosate by bacteria is a reasonable explanation for the question why there is a big discrepancy between real *in situ* bioavailability via biodegradation and dissolved glyphosate in soils under NaN_3 application.

6.9. Effect of herbicide glyphosate on soil respiration

The aim of this experiment was to study the effect of glyphosate on soil microbial respiration and to check whether the soil respiration is a parameter indicator for mineralization of glyphosate. The soil respiration in all 21 soils is intensive at the beginning and then reduces over the time. The soil respiration rates in soils during the first day can be argued as a ´´priming´´ effect produced by the mixing procedure making carbon, nutrients and oxygen more available in soils. Thus, the activity of the soil micro-flora in soils is stimulated at the very beginning time of the experiment (Salonils, 1978). Effects of glyphosate on soil respiration were classified into 3 groups: stimulating, depressing and no effects of glyphosate.

Lomanose, Kelheim, Neumarkt, Pear A20 and Feldkirchen are soils which had stimulating effect of glyphosate at the concentration of 10 µg glyphosate g^{-1} soil as compared to the control treatment which had no glyphosate application. This would mean that in these soils application of 10 µg glyphosate g^{-1} soil caused an enhancement on the microbial activity. This suggests that glyphosate may act as a nutrient source (C, N or P) for soil microorganisms in these soils. However, from literature, it has been suggested that glyphosate degradation is co-metabolic, not supporting microbial growth (Sprankle et al., 1975a). Therefore, it is not known if glyphosate stimulate soil respiration by supplying a source of C, or by supplying other nutrients enabling soil microorganisms to utilize other organic C sources in the soil or inducing a stress reaction. This result is consistent with findings of Carlisle and Trevors

(1986); Wardle and Parkinson (1990); Stratton and Stewart (1992); Araujo et al., 2003 who found that glyphosate at the concentration 10 and 100 times higher than recommended field rates stimulated soil respiration of forest soils and agricultural soils.

Out of the 21 soils examined, only 1 soil (Ada A02) showed a depressing effect of glyphosate on soil respiration. This shows direct toxic effect of glyphosate on soil microorganisms resulting from inhibition of aromatic amino acid biosynthesis. Thus, a direct toxic effect on respiration would be expected.

The other 15 soils (Apace-njiva, Berta A02, Brezje, Dunjar A06, Grace A13, Hanna A15, Joy A19, Konjise, Lamanose, Schyern-lysi, Zepovci, Skrinjar, Hohenwart, Lea A18 and Zepovci (Plitv.) showed no effect of glyphosate on soil respiration. This means that in these soils the soil respiration in both non treated variants and treated variants initially applied with 10 µg glyphosate g^{-1} soil was not different. This is consistent with study of Pereira et al. (2008) who showed that glyphosate had no effect on soil respiration at the concentration of 540 g of a.i. ha^{-1}.

The fact that no reasonable correlation between mineralization of glyphosate and soil respiration was found indicates that the degradation of glyphosate in soils does not correlate with a general microbial activity including soil perspiration. This fact is proven by previous studies (Rueppel et al., 1977; Wardle and Parkinson (1990); von Wiren-Lehr et al., 1997; Stenrod et al., 2005). This also shows that carbon dioxide production from soils after glyphosate application can not be related to the degradation of glyphosate in soil.

7. Conclusions and future perspectives

7.1. Conclusions

The major findings of this work can be summarized as follows:

- There is high variability in biomineralization of glyphosate in 21 different agricultural soils within 32 days of incubation. The bioavailability plays an important role on degradation of glyphosate in soil. Glyphosate is rapidly taken up by the microorganisms in the soil solution and the highest mineralization rate is reached shortly after application. The mineralization of glyphosate in soils depends on soil properties.

- Exchangeable H^+, soil pH, oxalate extractable Al^{3+} and bacterial cell numbers at the end of the experiments are identified as key soil parameters individually regulating the mineralization of glyphosate in soils whereas the mineralization glyphosate in soils is collectively controlled by exchangeable H^+, Ca^{2+} and K_2O.

- The dissolved glyphosate and desorbed amount of glyphosate (OECD) affect the mineralization of glyphosate in soils. No correlation between dissolved glyphosate (PW) and mineralization of glyphosate in soils was found. This is because of an artifact effect of high applied concentration of NaN_3 and the role of soil microorganisms on adsorption of glyphosate in soils.

- Both dissolved and desorbed glyphosate in soils are individually governed by exchangeable H^+ and soil pH. However, when having an interactive function of different soil parameters, the dissolved glyphosate using OECD is multiply regulated by soil pH, C % and silt, whereas desorbed glyphosate (OECD) is multiply controlled by exchangeable H^+, soil pH and Mg^{2+}.

- Extracted bacterial community from soil Feldkirchen could take up and degrade glyphosate in nutrient solution which have no additional C and P sources. The mineralization of glyphosate in nutrient solution is found to depend on bacterial cell numbers. The large amount of glyphosate taken up by microbes shortly after application could be mineralized in a long term period. This result reveals that in soil, bacterial uptake and sorption on abiotic particles are in competition for glyphosate.

- Effect of glyphosate application rate (10 µg glyphosate g^{-1} soil) on soil respiration depends on the type of soils. Fifteen out of 21 soils were found to have no effect of glyphosate on soil respiration. Only 1 out of 21 soils showed a depressing effect whereas 5 out of 21 soils showed a stimulating effect of glyphosate on soil respiration.

7.2. Future perspectives

Exchangeable H^+ is the most important parameter controlling the adsorption, desorption, formation of NaOH extractable residues, non extractable residues and mineralization of glyphosate in soil in this study. However, this is a new finding. Therefore, further studies can

be carried out to check whether the effect of the exchangeable H^+ depends on a direct effect on the microorganisms or on an effect on the bioavailability of glyphosate.

The soil and nutrient solution experiments show that the degradation of glyphosate by microbial community of soil Feldkirchen is effective. However, the degradation of glyphosate in soil Brejze is very low. Roughly 7 % of the initial glyphosate is mineralized within 32 days. This is explained by a very high adsorption capacity of glyphosate in this soil. However, the limiting factor for low degradation of glyphosate in soil Brezje have not been identified and verified yet. Thus, conducting a soil inoculation experiment in which soil Brezje is inoculated with an aliquot of soil Feldkirchen is necessary to see whether microbial parameter, particularly, glyphosate degrading microorganism or availability of glyphosate in soil solution is the most limiting factor explaining for the low degradation of glyphosate in this soil.

The multiple regression analysis for cumulative mineralization shows that Ca^{2+} and K_2O which have positive correlation with mineralization glyphosate are an important parameters regulating mineralization of glyphosate in soils. A resulting hypothesis is that (1) Ca^{2+}-Glyphosate complexes are more easily taken up by microbial cells than Glyplhosate alone and (2) K_2O can compete with glyphosate for sorption sites on soil. Therefore, further studies can be conducted in the laboratory to check these hypotheses.

References

Abdel-Mallek, A.Y., Abdel-Kader, M.I.A., Shonkeir, A.M., 2004. Effect of glyphosate on the fungal population, respiration and the decay of some organic matters in Egyptian soil. Microbiol. Res. 149, 69-73.

Abou-Assaf, N., Coats, J.R., 1987. Degradation of [^{14}C]isofenphos in soil in the laboratory under different soil pH'S, temperatures, and moistures**.** J. Environ. Sci. Health. Part B: Pesticides, Food Contaminants, and Agricultural Wastes. 22, 285-301.

Accinelli, C., Koskinen, W.C., Seebinger, J.D., Vicari, A., Sadowsky, M.J., 2005. Effects of incorporated corn residues on glyphosate mineralization and sorption in soil. J. Agric. Food. Chem. 53, 4110-4117.

Albers, C.N., Banta, G.T., Hansen, P.E., Jacobsen, O.S., 2009. The influence of organic matter on sorption and fate of glyphosate in soil–Comparing different soils and humic substances. Environ. Pollut. 157, 2865–2870.

Al-Rajab, A.J., Amellal, S., Schiavon, M., 2008. Sorption and leaching of ^{14}C-glyphosate in agricultural soils. Agron. Sustain. Dev. 28, 419–428.

Al-Rajab, A.J., Schiavon, M., 2010. Degradation of ^{14}C-glyphosate and aminomethylphosphonic acid (AMPA) in three agricultural soils. J. Environ. Sci. 22, 1374-1380.

Andrea, M.W., Peres, T. B., Luchini, L.C., Bazarin, S., Papini, S., Matallo, M.B., Savoy, V.L.T., 2003. Influence of repeated applications of glyphosate on its persistence and soil bioactivity. Pesq. Agropec. Bras. Brasília. 38, 1329-1335.

Araujo, A.S.F., Monteiro, R.T.R., Abarkeli, R.B., 2003. Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere. 52, 799–804.

Aubin, A. J., Smith, A. E., 1992. Extraction of 14 C Glyphosate from Saskatchewan soils. J. Agric. Food. 40, 1163-1165.

Autio, S., Siimes, K., Laitinen, P., Rämö, S., Oinonen, S., Eronen, L., 2004. Adsorption of sugar beet herbicides to Finnish soils. Chemosphere. 55, 215–226.

Baird, A.G., 2008. Chemical Prices. Growing the business of farming, FNSW farmers association.

Balthazor, T.M., Hallas, L.E., 1986. Glyphosate-degrading microorganisms from industrial activated sludge. Appl. Environ. Microbiol. 51, 432-434.

Barriuso, E., Benoit, P., Dubus, I.G., 2008. Formation of pesticide nonextractable (Bound) residues in soil: Magnitude, controlling factors and reversibility. Environ. Sci. Technol. 42, 1845–1854.

Baylis, A.D., 2000. Why glyphosate is a global herbicide: strengths, weaknesses and prospects. Pest. Manag. Sci. 56, 299-308.

Benachour, N., Seralini, G.E., 2009. Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. Chem. Res. Toxicol. 22, 97–105.

Betterton, E.A., 2003. Environmental fate of sodium azide derived from automobile airbags. Crit. Rev. Environ. Sci. Technol. 33, 423-458.

Bolognesi, C., Bonatti, S., Degan, P., Gallerani, E., Peluso, M., Rabboni, R., Roggieri, P., Abbondandolo, A., 1997. Genotoxic activity of glyphosate and its technical formulation Roundup. J. Agric. Food. Chem. 45, 1957-1962.

Bonfleur, E.J., Lavorenti, A., Tornisielo, V.L., 2011. Mineralization and degradation of glyphosate and atrazine applied in combination in a Brazilian Oxisol. J. Environ. Sci. and Health Part B. 46, 69–75.

Borggaard, O.K., Gimsing, A.L., 2008. Review - Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. Pest. Manag. Sci. 64, 441–456.

Botta, F., Lavison, G., Couturier, G., Alliot, F., Guigon, E.M., Fauchon, N., Guery, B., Chevreuil, M., Blanchoud, H., 2009. Transfer of glyphosate and its degradate AMPA to surface waters through urban sewerage systems. Chemosphere. 77, 133–139.

Brüsch, W., 2006. Glyphosate in small private water supply systems. Plant congress 2006.

Brust, G.E., 1990. Direct and indirect effects of four herbicides on the activity of Carabid beetles (Coleoptera: Carabidae). Pestic. Sci. 30, 309-320.

Buffin, D., Jewell, T., 2001. Health and environmental impacts of glyphosate: The implications of increased use of glyphosate in association with genetically modified crops. The Pesticide Action Network UK.

Burgess, J., Twigg, M.V., 2006. Iron: Inorganic & Coordination Chemistry. Inorg. Chem. John Wiley & Sons, Ltd, 1-45.

Busse, M.D., Ratcliff, A.W., Shestak, C.J., Powers, R.F., 2000. Non-target effects of glyphosate on soil microbes. Proceedings of the California Weed Sci. Soc. 52, 146-150.

Caetano, M.S., Ramalho, T.C., Botrel, D.F., Cunha, E.F.F., Mello, W.C., 2012. Understanding the inactivation process of organophosphorus herbicides: A DFT study of glyphosate metallic complexes with Zn^{2+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Co^{3+}, Fe^{3+}, Cr^{3+}, and Al^{3+}. J. Quant. Chem. Chemie.de, Ltd, 1-11.

Calvet, R., 1989. Adsorption of Organic Chemicals in Soils. Environ. Health. Persp. 83, 145-177.

Candela, L., Caballero, J., Ronen, D., 2010. Glyphosate transport through weathered granite soils under irrigated and non-irrigated conditions-Barcelona, Spain. Sci. Total Environ. 408, 2509-2516.

Carlisle, S., Trevors, J.T., 1986. Effect of the herbicide glyphosate on respiration and hydrogen consumption in soil. Water, Air, Soil Pollut. 27, 391-401.

Carlisle, S.M., Trevors, J.T., 1988. Glyphosate in the environment (Review article). Water, Air, Soil Pollut. 39, 409-420.

Castillo, L.F., Melgarejo, M.R., Piedrahita, C.L.F., Yunda, A.L., 2010. ^{14}C-glyphosate mineralization and follow up of the dynamics of *Pseudomonas* sp. populations in three soils under different uses in Tolima (Colombia). Agro. Colomb. 28, 413-420.

Cavalcante, D.G.S.M., Martinez, C.B.R., Sofia, S.H., 2008. Genotoxic effects of Roundup® on the fish *Prochilodus lineatus*. Mutat. Res. 655, 41–46.

Cavas, T., Könen, S., 2007. Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay. Mutagenesis. 22, 263–268.

Charnay, M.P., Mougin, C., Farrugia, A., Barriuso, E., 2004. Incorporation of pesticides by soil micro-organisms as a way of bound residues formation. Environ. Chem. Lett. 2, 27–30.

Cheah, U.B., Kirkwood, R.C., Lum, K.Y., 1998. Degradation of Four Commonly Used Pesticides in Malaysian Agricultural Soils. J. Agric. Food. Chem. 46, 1217-1223.

Cox, C., 1995. Glyphosate, Part 1: Toxicology. J. Pest. Ref. 15, 14-20.

Cruz, L.H., Santana, H., Zaia, C.T.B.V., Zaia, D.A.M., 2007. Adsorption of glyphosate on clays and soils from Paraná State: Effect of pH and competitive adsorption of phosphate. Braz. Arch. Biol. Technol. 50, 385-394.

Day, G.M., Hart, B.T., McKelvie, I.D., Beckett, R., 1997. Influence of natural organic matter on the sorption of biocides onto Goethite, II. Glyphosate. Environ. Technol. 18, 781-794.

Dick, R.E., Quinn, J.P., 1995. Glyphosate-degrading isolates from environmental samples: occurrence and pathways of degradation. Appl. Microbiol. Biotechnol. 43, 545-550.

Duke, S.O., Powles, S.B., 2008. Mini-review -glyphosate: a once-in-a-century herbicide. Pest. Manag. Sci. 64, 319–325.

Eberbach, P., 1998. Applying non-steady-state compartmental analysis to investigate the simultaneous degradation of soluble and sorbed glyphosate (*N*-(Phosphonomethyl)glycine) in four soils. Pestic. Sci. 52, 229-240.

Embacher, A., Zsolnay, A., Gattinger, A., Munch, J.C., 2007. The dynamics of water extractable organic matter (WEOM) in common arable topsoils: I. Quantity, quality and function over a three year period. Geoderma. 139, 11–22.

Ermakova, I.T., Kiseleva, N.I., Shushkova, T., Zharikov, M., Zharikov, G.A., Leontievsky, A.A., 2010. Bioremediation of glyphosate-contaminated soils. Appl. Microbiol. Biotechnol. 88, 585–594.

Eurostat., 2007. The use of plant protection products in the European Union - Data 1992-2003. European commission - Statistical books.

Feng, J.C., Thompsoni, D.G., 1990. Fate of glyphosate in a Canadian forest watershed. 2. Persistence in foliage and soils. J. Agric. Food. Chem. 38, 1118-1125.

Fitzgibbon, J., Braymey, H.D., 1988. Phosphate starvation induces uptake of glyphosate by *Pseudomonas* sp. strain PG2982. Appl. Environ. Microbiol. 54, 1886-1888.

Folberth, C., Scherb, H., Suhadolc, M., Munch, J.C., Schroll, R., 2009a. In situ mass distribution quotient (iMDQ)–A new factor to compare bioavailability of chemicals in soils? Chemosphere. 75, 707–713.

Folberth, C., Suhadolc, M., Scherb, H., Munch, J.C., Schroll, R., 2009b. Batch experiments versus soil pore water extraction – What makes the difference in isoproturon (bio)availability? Chemosphere. 77, 756–763.

Forlani, G., Mangiagalli, A. Nielsen, E., Suardi, C.M., 1999. Degradation of the phosphonate herbicide glyphosate in soil: evidence for a possible involvement of unculturable microorganisms. Soil Biol. Biochem. 31, 991-997.

Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M.C., Seralini, G.E., 2009. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicol. 262, 184–191.

Getenga, Z.M., Kengara, F.O., 2004. Mineralization of glyphosate in compost-amended soil under contaminated conditions. Bull. Environ. Contam. Toxicol. 72, 266-275.

GEUS., 2001. Groundwater survey 2001. Part 5: Pesticides and degradation products. The geological survey of Denmark and Greenland ministry of environment and energy.

Gianessi, L.P., 2008. Review - economic impacts of glyphosate-resistant crops. Pest. Manag. Sci. 64, 346–352.

Giesy, J.P., Dobson, S., Solomon, K.R., 2000. Ecotoxicological risk assessment for Roundup herbicide. Rev. Eviron. Contam. Toxicol. 167, 35-120.

Gimsing, A.L., Borggaard, O.K., Jacobsen, O.T., Aamand, J., Sorensen, J., 2004a. Chemical and microbiological soil characteristics controlling glyphosate mineralization in Danish surface soils. Appl. Soil Ecol. 27, 233–242.

Gimsing, A.L., Borggaard, O.K., Bang, M., 2004b. Influence of soil composition on adsorption of glyphosate and phosphate by contrasting Danish surface soils. Europ. J. Soil Sci. 55, 183–191.

Gimsing, A.L., Szilas, C., Borggaard, O.K., 2007. Sorption of glyphosate and phosphate by variable-charge tropical soils from Tanzania. Geoderma. 138, 127–132.

Glass, R.L., 1987. Adsorption of glyphosate by soils and clay minerals. J. Agric. Food. Chem. 35, 497-500.

Goodwin, M., 2010. The Science of making your glyphosate application work harder. Dow. Agro. Sci.

Grundmann, S., Doerfler, U., Munch, J.C., Ruth, B., Schroll, R., 2011. Impact of soil water regime on degradation and plant uptake behaviour of the herbicide isoproturon in different soil types. Chemosphere. 82, 1461–1467.

Hance, R.J., 1976. Adsorption of glyphosate by soils. Pestic. Sci. 7, 363-366.

Haney, R.L., 2000. Effect of glyphosate on soil microbial activity and biomass. Weed Sci. 48, 89-93.

Hansen, R.S., Holm, P.E., Jacobsen, O.S., Jacobsen, C.S., 2004. Sorption, mineralization and mobility of *N*-(phosphonomethyl)glycine (glyphosate) in five different types of gravel. Pest. Manag. Sci. 60, 570–578.

Harrison, S.A., 1998. The Fate of Pesticides in the Environment. Agrichemical fact sheet. College of Agricultural Sciences. Cooperative Extension, The Pennsylvania State University.

Haughton, A.J., Bell, J.R., Boatman, N.D., Wilcox, A., 2001. The effect of the herbicide glyphosate on non-target spiders: Part II. Indirect effects on *Lepthyphantes tenuis* in field margins. Pest. Manag. Sci. 57, 1037-1042.

Hensley, D.L., Beuerman, D.S.N., Carpenter, P.L., 1978. The inactivation of glyphosate by various soils and metal salts. Weed Res. 18, 287-291.

Horth, H., 2010. Monitoring results for surface water and groundwater. European glyphosate environmental information source (egeis).

Huang, W., Weber, Jr.J.Jr., Yu, H., 1998. Hysteresis in the sorption and desorption of hydrophobic organic contaminants by soils and sediments 2. Effects of soil organic matter heterogeneity. J. Contam. Hydrol. 31, 149–165.

Ilstedt, U., Nordgren,A., Malmer, A., 2000. Optimum soil water for soil respiration before and after amendment with glucose in humid tropical acrisols and a boreal mor layer. Soil Biol. Biochem. 32, 1591-1599.

Jacob, G.S., Garbow, J.R., Hallas, L.E., Kimack, N.M., Kishore, G.M., Schaffer, J., 1988. Metabolism of glyphosate in Pseudomonas sp. Strain LBr. Appl. Environ. Microbiol. 54, 2953-2958.

Jensen, L.C., Gan, J., Baez, M., Fuentes, R., Escudey, M., 2009. Adsorption of Glyphosate on Variable-Charge, Volcanic Ash–Derived Soils. J. Environ. Qual. 38, 1449–1457.

Jjaer, J., Olsen, P., Barlebo, H.C., Henriksen, T., Plauborg, F., Grant, R., Nygaard, P., Gudmundsson, L., Rosenbom, A., 2006. The Danish pesticide leaching assessment program. Geological survey of Denmark and Greenland.

Jonge, H., Jonge, L.W., 1999. Influence of pH and solution composition on the sorption of glyphosate and prochloraz to sandy loam soil. Chemosphere. 39, 753-763.

Kah, M., Brown, C.D., 2007. Changes in pesticide adsorption with time at high soil to solution ratios. Chemosphere. 68, 1335–1343.

Kengara, F.O., 2010. Enhancement of degradation of DDT and HCB in tropical clay soils in model experiments. PhD thesis at the Department of Soil Ecology, Center of Life and Food Sciences, Technical University of Munich. Freising-Weihenstephan, Germany.

Kim, B., Kim, Y.S., Kim, B.M., Hay, A.G., Mcbride, M.B., 2011. Effect of soil metal contamination on glyphosate mineralization: Role of zinc in the mineralization rates of two copper-spiked mineral soils. Environ. Toxicol. Chem. 30, 596–601.

Kishore, G.M., Jacob, G.S., 1987. Degradation of glyphosate by *Pseudomonas* sp. PG2982 via a sarcosine intermediate. J. Biol. Chem. 262, 12164-12168.

Kjaer, J., Olsen, P., Ullum, M., Grant, R., 2005. Leaching of glyphosate and amino-methylphosphonic acid from Danish agricultural field sites. J Environ. Qual. 34, 608-620.

Klier, C., 2007. Environmental fate of the herbicide glyphosate in the soil-plant system: Monitoring and modelling using large-scale weighing lysimeters. PhD thesis at the Department of Soil Ecology, Center of Life and Food Sciences, Technical University of Munich. Freising-Weihenstephan, Germany.

Kogan, M., Metz, A., Ortega, R., 2003. Adsorption of glyphosate in Chilean soils and its relationship with unoccupied phosphate binding sites. Pesq. Agropec. Bras. Brasilia. 38, 513-519.

Kolawole, O.A., Akinsoji, A.O., 2011. Biodegradation of glyphosate pesticide by bacteria isolated from agricultural soil. Rep. Opin. 3, 124-128.

Kools, S.A.E., van Roovert, M., van Gestel, C.A.M., van Straalen, N.M., 2005. Glyphosate degradation as a soil health indicator for heavy metal polluted soils. Soil Biol. Biochem. 37, 1303–1307.

Kremera, R.J., Means, N.E., 2009. Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. Europ. J. Agron. 31, 153–161.

Laitinen, P., 2009. Fate of the organophosphate herbcide glyphosate in arable soils and it´s relationship to soil phosphorous status. Doctoral dissertation at the Department of environmental science, University of Kuoplo, Finland.

Laitinen, P., Rämö, S., Nikunen, U., Jauhiainen, L., Siimes, K., Turtola, E., 2009. Glyphosate and phosphorus leaching and residues in boreal sandy soil. Plant Soil. 323, 267–283.

Lancaster, S.H., Hollister, E.B., Senseman, S.A., Gentry, T.J., 2010. Effects of repeated glyphosate applications on soilmicrobial community composition and the mineralization of glyphosate. Pest. Manag. Sci. 66, 59–64.

Landry, D., Dousset, S., Fournier, J.C., Andreux, F., 2005. Leaching of glyphosate and AMPA under two soil management practices in Burgundy vineyards (Vosne-Romanee, 21-France). Environ. Pollut. 138, 191-200.

Langiano, V.C., Martinez, C.B.R., 2008. Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*. Comp. Biochem. Physiol. Part C. 147, 222–231.

Lerbs, W., Stock, M., Parthier, B., 1990. Physiological aspects of glyphosate degradation in *Alcaligenes spec.* strain GL. Arch. Microbiol. 153, 146-150.

Levesque, C.A., Rahe, J.E., 1992. Herbicide interections with fungal rootphathogens, with special reference to glyphosate. Annu. Rev. Phylopalhoi. 30, 579-602.

Lindsay, E.A., French, K., 2004. The impact of the herbicide glyphosate on leaf litter invertebrates within Bitou bush, *Chrysanthemoides monilifera* ssp *rotundata*, infestations. Pest. Manag. Sci. 60, 1205–1212.

Liu, C.M., McLean, P.A., Sookdeo, C.C., Cannon, F.C., 1991. Degradation of the herbicide glyphosate by members of the family Rhizobiaceae. Appl. Environ. Microbiol. 57, 1799-1804.

Loganathan, V.A., 2006. Effects of sorption and desorption on bioavailability of Atrazine in soils amended with crop residue derived char. Department of Crop, Soil and Environmental Sciences, University of Arkansas, India.

Luijendijk, C.D., Beltman, W.H.J., Smidt, R.A., van der Pas, L.J.T., Kempenaar, C., 2005. Measures to reduce glyphosate runoff from hard surfaces. 2. Effect of time interval between application and first precipitation event. Plant research international B.V., Wageningen.

Luijendijk, C.D., Beltman, W.H.J., Wolters, M.F., 2003. Measures to reduce glyphosate runoff from hard surfaces. 1. Effect of a bufferzone around the drain. Plant research international B.V., Wageningen.

Lupwayi, N.Z., Harker, K.N., Clayton, G.W., O´Donovan, J.T., Blackshaw, R.E., 2009. Soil microbial response to herbicides applied to glyphosate-resistant canola. Agric. Ecos. Environ. 129, 171–176.

Mamy, L., Barriuso, E., 2005. Glyphosate adsorption in soils compared to herbicides replaced with the introduction of glyphosate resistant crops. Chemosphere. 61, 844–855.

Martinez, A., Reyes, I., Reyes, N., 2007. Cytotoxicity of the herbicide glyphosate in human peripheral blood mononuclear cells. Biomédica. 27, 594-604.

McAuliffe, K.S., Hallas, L.E., Kulpa, C.F., 1990. Glyphosate degradation by *Agrobacterium radiobacter* isolated from activated sludge. J. Indust. Microbiol. 6, 219-221.

McConnell, J.S., Hossner, L.R., 1985. pH-Dependent adsorption isotherms of glyphosate. J. Agric. Food. Chem. 33, 1075-1078.

Mekwatanakarn, P., Sivasithamparam, K., 1987. Effect of certain herbicides on soil microbial populations and their influence on saprophytic growth in soil and pathogenicity of take-all fungus. Biol. Fertil. Soils. 5, 175-180.

Meyer-Aurich, A., Matthes, U., Osinski, E., 2001. Intergrating sustainability in agriculture-trade-offs and economic consequences demonstrated with a farm model in Bavaria. Department of Economics and Social Sciences and Department of Plant Science at the Center of Life and Food Sciences, Technical University of Munich. Freising-Weihenstephan, Germany.

Miles, C.J., Moye, H.A., 1988. Extraction of glyphosate herbicide from soil and clay minerals and determination of residues in soils. J. Agric. Food. Chem. 1088, 488-491.

Moneke, A.N., Okpala, G.N., Anyanwu, C.U., 2010. Biodegradation of glyphosate herbicide *in vitro* using bacterial isolates from four rice fields. Afr. J. Biotechnol. 9, 4067-4074.

Monsanto., 1996. Glyphosate fact sheet. In Pesticides News No.33, 28-29.

Morillo, E., Undabeytia, t., Maqueda, C., Ramos, A., 2000. Glyphosate adsorption on soils of different characteristics. Influence of copper addition. Chemosphere. 40, 103-107.

Moshier, L.J., Penner, D., 1978. Factors influencing microbial degradation of ^{14}C-Glyphosate to $^{14}CO_2$ in soil. Weed Sci. 26, 686-691.

Ngigi, A., Dörfler, U., Scherb, H., Getenga, Z., Boga, H., Schroll, R., 2011. Effect of fluctuating soil humidity on *in situ* bioavailability and degradation of atrazine. Chemosphere. 84, 369–375.

Nomura, N.S., Hilton, H.W., 1977. The adsorption and degradation of glyphosate in five Hawaiian sugarcane soils. Weed Res. 17, 113-121.

OECD., 2000. OECD guideline 106–OECD guideline for the testing of chemicals: adsorption–desorption using a batch equilibrium method.

ÖNORM L 1087., 1993. Bestimmung von pflanzenverfu¨gbarem Phosphat und Kalium nach der Calcium-Acetat-Lactat (CAL) – Methode. Österreichisches Normungsinstitut, Wien.

Parochetti, J.V., Warren, G.F., 1970. Behavior of potassium azide in the soil. Weed Sci. 18, 555-560.

Partoazar, M., Hoodaji, M., Tahmourespour, A., 2011. The effect of glyphosate application on soil microbial activities in agricultural land. Afr. J. Biotechnol. 10, 19419-19424.

Pereira, J.L., Picanco, M.C., Silva, A.A., Santos, E.A., Tome, H.V.V., Olarte, J.B., 2008. Effects of glyphosate and endosulfan on soil microorganisms in soybean crop. Planta Daninha, Vicosa-MG. 26, 825-830.

Peruzzo, P.J., Porta, A.A., Ronco, A.E., 2008. Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in north pampasic region of Argentina. Environ. Pollut. 156, 61-66.

Piccolo, A., Celano, G., Arienzo, M., Mirabella, A., 1994. Adsorption and desorption of glyphosate in some European soils. J. Environ. Sci. Health. B29, 1105-1115.

Piccolo, P., Celano, G., Pietramellara, G., 1992. Adsorption of the herbicide glyphosate on a metal-humic acid complex. The Sci. Total Environ. 123, 77-82. Elsevier Science Publishers B.V., Amsterdam.

Pipke, R., Armhein, N., 1988. Degradation of the phosphonate herbicide glyphosate by *Arthrobacter atrocyaneus* ATCC 13752. Appl. Environ. Microbiol. 54, 1293-1296.

Pipke, R., Schulz, A., Amrhein, N., 1987. Uptake of Glyphosate by an *Arthrobacter* sp. Appl. Environ. Microbiol. 53, 974-978.

Prata, F., Cardinali, V.C.B., Lavorenti, A., Tornsielo, V.L., Regitano, J.B., 2003. Glyphosate sorption and desorption in soils with distinct phosphorous levels. Scientia. Agricola. 60, 175-180.

Rafiei Keshteli, M., Farahbakhsh, M., Savaghebi, G.R., 2011. Adsorption behavior of glyphosate in some citrus garden soils of Iran. J. Environ. Agri. Food. Chem. 10, 1943-1951.

Ratcliff, A.W., Busse, M.D., Shestak, C.J., 2006. Changes in microbial community structure following herbicide (glyphosate) additions to forest soils. Appl. Soil Ecol. 34, 114–124.

Reddy, K.N., Rimando, A.M., Duke, S.O., Nandula, V.K., 2008. Aminomethylphosphonic acid accumulation in plant species treated with glyphosate. J. Agric. Food Chem. 56, 2125–2130.

Relyea, R.A., 2005a. The lethal impact of Roundup on aquatic and terrestrial amphibians. Ecol. Appl. 15, 1118–1124.

Relyea, R.A., 2005b. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. Ecol. Appl. 15, 618–627.

Richard, S., Moslemi, S., Sipahutar, H., Benachour, N., Seralini, G.E., 2005. Differential Effects of Glyphosate and Roundup on Human Placental Cells and Aromatase. Environ. Health Persp. 113, 716-720.

Roy, D.N., Konar, S.K., Banerjee, S., Charles, D.A., 2004. Persistence, movement, and degradation of glyphosate in selected Canadian boreal forest soils. J. Agric. Food. Chem. 37, 437-440.

Rueppel, M.L., Brightwell, B.B., Schaefer, J., Marvel, J.T., 1977. Metabolism and Degradation of glyphosate in soil and water. J. Agric. Food. Chem. 25, 517-528.

Salonius, P.O., 1978. Effects of mixing and various temperature regimes on the respiration of fresh and air-dried coniferous raw humus materials. Soil Biol. Biochem. 10, 479-482.

Savitz, D.A., Arbuckle, T., Kaczor, D., Curtis, K.M., 1997. Male Pesticide Exposure and Pregnancy Outcome. Am. J. Epidemiol. 146, 1025-1036.

Schroll, R., Becher, H.H., Dörfler, U., Gayler, S., Grundmann, S., Hartmann, H.P., Rouss, J., 2006. Quantifying the effect of soil moisture on the aerobic microbial mineralization of selected pesticides in different soils. Environ. Sci. Technol. 40, 3305-3312.

Schroll, R., Brahushi, F., Dörfler, U., Kühn, S., Fekete, J., Munch, J.C., 2004. Biomineralization of 1,2,4-trichlorobenzene in soils by an adapted microbial population. Environ. Pollut. 127, 395–401.

Schuette, J., 1998. Environmental fate of glyphosate. Environmental monitoring and pest management, Department of pesticide regulation, Sacramento, CA 95824-5624.

Schütte, G., Mertens, M., 2010. Potential effects of the introduction of a sugar beet variety resistant to glyphosate on agricultural practice and on the environment. BfN-Skripten 277-2010 international year of biodiversity-Federal Agency of nature and conservation.

Screpanti, C., Accinelli, C., Vicari, A., Catizone, P., 2005. Glyphosate and glufosinate-ammonium runoff from a corn-growing area in Italy. Agron. Sustain. Dev. 25, 407–412.

Scribner, E.A, Battaglin, W.A., Dietze, J.E., Thurman, E.M., 2003. Reconnaissance data for glyphosate, other selected herbicides, their degradation products, and antibiotics in 51 streams in nine midwestern States, 2002. U.S. Department of the Interior, U.S. Geological Survey.

Scribner, E.A., Battaglin, W.A., Gilliom, R.J., Meyer, M.T., 2007. Concentrations of glyphosate, it´s degradation product, aminomethyphosphonic acid, and glufosinate in ground-and surface-water, rainfall, and soil samples collected in the United States, 2001-2006. U.S. Department of the Interior, U.S. Geological Survey.

Sheals, J., Sjöberg, S., Persson, P., 2002. Adsorption of glyphosate on Goethite: Molecular characterization of surface complexes. Environ. Sci. Technol. 36, 3090-3095.

Shinabarger, P.L., Schmitt, E.K., Braymer, H.D, Larson, A.D., 1984. Phosphonate utilization by the glyphosate-degrading *Pseudomonas* sp. strain PG 2982. Appl. Environ. Microbiol. 48, 1049-1050.

Shoval, S., Yariv, S., 1979. The interaction between Rounup (glyphosate) and montmorillonite. Part I. Infrared study of the sorption of glyphosate by montmorillonite. Clays and Clay Minerals. 27, 19-28.

Shushkova, T., Ermakova, I., Leontievsky, A., 2009. Glyphosate bioavailability in soil. Biodegradation. 21, 403–410.

SIST ISO 10390., 1996. Soil quality: Determination of pH, 5 p.

SIST ISO 10694., 1996. Determination of organic and total carbon after dry combustion (elementary analysis), 8 p.

SIST ISO 13878., 1998. Soil quality: Determination of total nitrogen content by dry combustion ("elemental analysis"), 5p.

SIST ISO 14235-Modification after Walkely-Black., 1998. Soil quality: Determination of organic carbon by sulfochromic oxidation, 5p.

Smith, A.E., Aubin, A.J., 1993. Degradation of 14C-Glyphosate in Saskatchewan Soils. Bull. Environ. Contam. Toxicol. 50, 499-505.

Soil Survey Laboratory Methods Manual., 1992. United States department of agriculture, Soil conservation service, National soil survey center, 400 p.

Sorensen, S.R., Schultz, A., Jacobsen, O.S., Aamand, J., 2006. Sorption, desorption and mineralisation of the herbicides glyphosate and MCPA in samples from two Danish soil and subsurface profiles. Environ. Pollut. 141, 184-194.

Sprankle, P., Meggitt, W. F., Penner, D., 1975a. Adsorption, mobility, and microbial degradation of glyphosate in the soil. Weed Sci. 23, 229-234.

Sprankle, P., Meggitt, W.F., Penner, D., 1975b. Rapid inactivation of glyphosate in the soil. Weed Sci. 23, 224–228.

Stenrod, M., Eklo, O.M., Charnay, M.P., Benoit, P., 2005. Effect of freezing and thawing on microbial activity and glyphosate degradation in two Norwegian soils. Pest. Manag. Sci. 61, 887–898.

Stratton, G.W., Stewart, K.E., 1992. Glyphosate effects on microbial biomass in a coniferous forest soil. Environ. Toxicol. Water Qual. 7, 223-236.

Susnik, A., Pogacar, T., Gregoric, G., Roskar, J., Ceglar, A., 2010. Establishment of agricultural drought monitoring at different spatial scales in southeastern Europe. Acta. Agric. Slov. 95, 231-243.

Sviridov, A.V., Zelenkova, N.F., Vinokurova, N.G., Ermakova, I.T., Leontievsky, A.A., 2011. New approaches to identification and activity estimation of glyphosate degradation enzymes. Biochem. Moscow. 76, 720-725.

Talbot, H.W., Johnson, L.M., Munneckew, D.M., 1984. Glyphosate utilization by *Pseudomonas* sp. and *Alcaligenes* sp. Isolated from environmental sources. Curr. Microbiol. 10, 255-260.

Tharp, C., 2012. Minimizing pesticide contaminated soil around the home and garden. The U.S. Department of Agriculture (USDA), Montana State University and Montana State University Extension.

Torstensson, L., 1985. Behaviour of glyphosate in soils and its degradation. In: Grossbard, E., Atkinson, D (eds). The Herbicide Glyphosate. Butterworths, London, 137-150.

Torstensson, L., Börjesson, E., Stenström, J., 2005. Efficacy and fate of glyphosate on Swedish railway embankments. Pest. Manag. Sci. 61, 881–886.

Traas, T.P., Smit, C.E., 2003. Environmental risk limit for aminomethylphosphonic acid (AMPA). National Institute of Public Health and the Environment, The Netherlands.

Trevors, J.T., 1996. Sterilization and inhibition of microbial activity in soil. J. Microbiol. Met. 26, 53-59.

Tsui, M.T.K., Chu, L.M., 2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. Chemosphere. 52, 1189–1197.

Tu, M., Hurd, C., Robison, R., Randall, J.M., 2001. Glyphosate. Weed Control Methods Handbook, The Nature Conservancy.

USDA., 1997. Glyphosate herbicide information profile. United States, Department of Agriculture, Forest Service Pacific Northwest Region.

USEPA., 1993. Glyphosate. Prevention, pesticides and toxic substances. EPA. R.E.D. Facts.

VDLUFA, Methodenbuch Band I 4. Auflage., 1991. Die Untersuchung von Böden.

Veiga, F., Zapata, J.M., Marcos, M.L.F., Alvarez, E., 2001. Dynamics of glyphosate and aminomethylphosphonic acid in a forest soil in Galicia, north-west Spain. Sci. Total Environ. 271, 135-144.

Vera, M.S., Lagomarsino, L., Sylvester, M., Perez, G.L., Rodrıguez, P., Mugni, H., Sinistro, R., Ferraro, M., Bonetto, C., Zagarese, H., Pizarro, H., 2010. New evidences of Roundup (glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. Ecotoxicol. 19, 710–721.

Vereecken, H., 2005. A review: Mobility and leaching of glyphosate. Pest. Manag. Sci. 61, 1139–1151.

Wackett, L.P., Shanes, S.L., Venditti, C.P., Walsh, C.T., 1987. Bacterial carbon-phosphorus lyase: products, rates, and regulation of phosphonic and phosphinic acid metabolism. J. Bacteriol. 169, 710-717.

Wang, Y.J., Zhou, D.M., Sun, R.T., 2005. Effect of phosphate on the adsorption of glyphosate on 3 different types of Chinese soils. J. Environ. Sci. 17, 711-715.

Wardle, D.A., Parkinson, D., 1990. Effects of three herbicides on soil microbial biomass and activity. Plant and Soil. 122, 21-28.

Watts, M., 2009a. Glyphosate. Empowering people for change. Pesticide action network Asia and the Pacific.

Watts, M., 2009b. Roundup's not OK. Pesticide action network Asia and the Pacific.

Wauchope, R.D., Yeh, S., Linders, J.B.H.J., Kloskowski, R., Tanaka, K., Rubin, B., Katayama, A., Kördel, W., Gerstl, Z., Lane, M., Unsworth, J.B., 2002. Review –

pesticide soil sorption parameters: theory, measurement, uses, limitations and reliability. Pest. Manage. Sci. 58, 419–445.

Wauchope, R.D., Buttler, T.M., Hornsby, A.G., Augustijn-Beckers, P.W., Burt, J.P., 1992. The SCS/ARS/CES pesticide properties database for environmental decision-making. Rew. Eviron. Contam. Toxicol. 123, 1-155.

Weaver, M.A., Krutz, L.J., Zablotowicz, R.M., Reddy, K.N., 2007. Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil. Pest. Manag. Sci. 63, 388–393.

WHO., 1994. Environmental health criteria 159-Glyphosate. International programme on chemical safety.

WHO., 2005. Glyphosate and AMPA in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality.

Wiren-Lehr, S., Komoßa, D., Gläßgen, W.E., Sandermann, H., Scheunert, I., 1997. Mineralization of [^{14}C]glyphosate and its plant-associated residues in arable soils originating from different farming systems. Pest. Sci. 51, 436-442.

Wolf, D.C., Dao, T.H., Scott, H.D., Lavy, T.L., 1989. Influence of sterilization methods on selected soil microbiological, physical, and chemical properties. J. Environ. Qual. 18, 39-44.

Woodburn, A.T., 2000. Glyphosate: production, pricing and use worldwide. Pest. Manag. Sci. 56, 309-312.

Worrall, F., Fernandez-Perez, M., Johnson, A.C., Flores-Cesperedes, F., Gonzalez-Pradas, E., 2001. Limitations on the role of incorporated organic matter in reducing pesticide leaching. J. Contam. Hydrol. 49, 241–262.

Yu, Y., Zhou, Q.X., 2005. Adsorption characteristics of pesticides methamidophos and glyphosate by two soils. Chemosphere. 58, 811–816.

Zablotowicz, R., Accinelli, C., Krutz, L.J., Reddy, K., 2009. Soil depth and tillage effects on glyphosate degradation. J. Agric. Food. Chem. 57, 4867–4871.

Zhang, P., Sheng, G., Wolf, D.C., Feng, Y., 2004. Reduced biodegradation of benzonitrile in soil containing wheat-residue-derived ash. J. Environ. Qual. 33, 868-872.

Zhou, D.M., Wang, Y.J., Cang, L., Hao, Z., Luo, X.S., 2004. Adsorption and cosorption of cadmium and glyphosate on two soils with different characteristics. Chemosphere. 57, 1237–1244.