Influence of Soil Properties and Organic Pesticides on Soil Microbial Metabolism

Ylva Schnürer
Faculty of Forest Sciences
Department of Forest Ecology
Umeå

Doctoral thesis
Swedish University of Agricultural Sciences
Umeå 2006

Acta Universitatis Agriculturae Sueciae
2006: 118

ISSN 1652-6880
ISBN 91-576-7267-9

Tryck: Arkitektkopia, Umeå 2006

Abstract

Schnürer, Y., 2006. *Influence of soil properties and organic pesticides on soil microbial metabolism.* Doctor's dissertation.
ISSN 1652-6880, ISBN 91-576-7267-9.

In many areas of the world undesirable dispersal of organic pesticides into untargeted environments has occurred, and is continuing, raising serious concerns about their potential impact on the environment and human health. The main agents ultimately responsible for the degradation of these substances are microorganisms, so it is important to understand their effects on microbial metabolism and the complex interactive effects of soil parameters, microbial activities, pesticide availability and pesticide degradation dynamics. The studies presented in this thesis elucidate some such interactions involving three pesticides: 2,6-dichlorobenzamide, 2,6-dichlorobenzonitrile and 2,4-dichlorophenol. The respiration kinetics of soil microorganisms in the presence and absence of pesticides were measured and used to derive information on catabolic and anabolic components of the microbial responses. Furthermore, the effects of charge density and pH of surfaces of soil particles were also investigated by manipulating soil solution pH, the pH of organic matter surfaces and the mineral composition (and hence surface charge density) of test soils. The pH and charge density of particle surfaces were found to strongly influence soil microbial processes and the fate and behaviour of pesticides in soils. In addition, the effects of sorption on the availability and degradability of glyphosate, one of the most commonly used pesticides in the world, were examined using respiration measurements in combination with attenuated total reflectance-Fourier transform infra-red spectroscopy analysis. Raising the pH of an acidic soil with high organic matter content was found to reduce the toxic effects of 2,4-dichlorophenol on anabolic microbial processes, but the effects of its toxicity towards catabolic processes were less pronounced. 2,6-dichlorobenzamide had greater negative effects on microbial metabolic processes at neutral than at acid pH. Increasing the surface charge density and raising the surface pH of the soil organic matter reduced the negative effects of 2,6-dichlorobenzonitrile on microorganisms. Soil surface charge density and pH therefore interactively influence the effects of the pesticides on microbial metabolism. Further experiments showed that sorbed glyphosate can be used by the microorganisms as a source of C, N and possibly P.

Key words: Soil surface charge; Soil surface pH; Sorption; Pesticide; Dichlobenil; BAM; 2,4-DCP; Glyphosate Respiration kinetics; SIR; Microbial metabolism; Catabolism; Anabolism

Contents

Appendix

Paper I-III

This doctoral thesis is based upon the following papers, hereafter referred to by the corresponding Roman numerals:

I. Schnürer, Y., Nordgren, A., Nilsson, M., 2006. Effects of 2,6-dichlorobenzamide (BAM), 2,6-dichlorobenzonitrile (dichlobenil) and 2,4-DCP on soil microbial metabolism. Submitted

II. Schnürer, Y., Skyllberg, U., Nilsson, M., 2006. Soil microbial responses to organic pesticides vary with soil surface charges and soil surface pH. Manuscript

III. Schnürer Y., Persson P., Nilsson M., Nordgren A., Giesler R. 2006. Effect of surface sorption on microbial degradation of glyphosate. Environmental Science & Technology 40, 4145-4150

Abbreviations

AMPA	Aminomethylphosphonic acid
ATR-FTIR	Attenuated total reflectance-Fourier transform infra-red spectroscopy
BAM	2,6-Dichlorobenzamide
BR	Basal respiration rate
BR_{diff}	Difference in basal respiration before and after pesticide addition
DCP	Dichlorophenol
K_{OW}	Octanol water partitioning coefficient
MLR	Multiple linear regression
OM	Organic matter
PCA	Principal component analysis
PCR	Principal component regression
PLS	Partial least squares projection to latent structures
PZC	Point of zero charge
R_{max}	Maximum respiration rate
SCD	Surface charge density
SIR	Substrate-induced respiration
SOM	Soil organic matter
t_{max}	Time to reach maximum respiration rate
μ	Natural logarithm of exponential growth

Introduction

The focus of this thesis is on the importance of soil properties for the activities of soil microorganisms, especially their responses to pesticides. The thesis addresses the effects of interactions between soil particle surfaces, pH and pesticides on the metabolism of soil microorganisms. More specifically, the main issues addressed were the effects of variations in surface charge density (SCD) and pH in both soil solution and at the surface of the soil organic matter (SOM) in the presence and absence of organic pesticides on catabolic and anabolic processes of microbial metabolism. The effects of surface sorption on the degradability and bioavailability of an organic pesticide in soil were also addressed. In order to assess the effect of the soil properties and pesticides on the microorganisms, respiration kinetic measurements that distinguished between catabolic and anabolic components of microbial metabolic responses were used.

Pesticides – for better and worse

In daily life people are exposed to a variety of harmful synthetic compounds that have been released into the environment through both commercial and domestic activities. These compounds include pesticides used for a variety of purposes, *inter alia*; to increase the durability of wooden products, to control weeds in gardens or fields, and to protect crops. The extensive use of pesticides, which must be toxic (at least to the targeted pests) to be effective, has led to the unwanted dispersal of pesticides into untargeted environments (Clausen et al., 2002, Droz et al., 2005). The ideal pesticide acts on the target organism, degrades into harmless metabolites and disappears before it has had time to migrate into and adversely affect non-targeted environmental compartments. However, pesticides are designed to persist long enough to have the desired effects on the target organisms. Moreover, pesticide metabolites also vary in terms of persistency and toxicity (e.g. Greer et al., 1990). Furthermore, their metabolites often have very different physicochemical characteristics in terms of their sorption and water solubility parameters (e.g. Barja & Alfonso, 2005, Clausen et al., 2004). These features highlight the importance of understanding pesticide degradation processes and the effects of the pesticides on microbial metabolism.

In order to develop efficacious pesticides and application techniques that minimize dispersal of xenobiotic chemicals into untargeted environments, and remediation strategies, it is important to understand the effects of both the pure compounds and their degradation products on microbial activity in soils. Microbial degradation and transformation of pesticides occurs through oxidation, reduction, hydrolytic and synthetic reactions and, in common with the decomposition of naturally occurring recalcitrant compounds, there are facilitative interactions within the microbial community (Bollag & Liu, 1990). Numerous microbial species are known to have the capacity to degrade complex organic structures. Important groups of soil organisms in this respect include actinomycetes (Paul & Clark, 1996) and many fungi of various classes: ascomycota, basidiomycota,

deutoeromycota and zygomycota (Cerniglia et al., 1992). For successful degradation of any compound the microorganisms must be able to produce appropriate enzymes and the compound must not be too toxic to them. The compound also needs to be bioavailable, i.e. accessible to the microorganisms and not irreversibly sorbed to soil particles. The sorption parameters of the compounds are governed by their hydrophobicity and the physicochemical conditions of the soil.

Parameters of the soil environment

Interactions with microorganisms

Two physicochemical variables of the soil environment that strongly influence microbial processes are the soil particle surface charge density (SCD) and the pH. Both SCD and pH are highly influenced by the composition of soil particles (i.e. mineral and organic components) and their particle size distribution (Sposito, 1989; Smith et al., 1993).

The soil surface charge is of great significance for microbial adhesion (Mills, 2003). In addition, growth on a surface is highly advantageous for microorganisms since surfaces can attract nutrients and organic compounds (Stark et al., 1938). Other processes that are also affected by the sorption properties of soil surfaces include (*inter alia*) exoenzyme activities (Ahn et al., 2002, George et al., 2005) and the physical availability of water, i.e. the thickness of the water films on them, and how strongly the films are bound.

The pH and charge at the surface of a soil particle are intimately related to the pH and ionic strength of the bulk solution (Bolan et al. 1999). The abundance of protons in the diffusive double layer generates the pH at the soil particle surface. The diffusive double layer is formed by the force of attraction of the negatively charged particle surfaces in the soil on the cations in the soil solution. The point of zero charge (PZC) of microorganisms and most soil particle surfaces is lower than the soil solution pH, which is usually 5-8 (Mills, 2003). Thus, microorganisms and soil surfaces are generally negatively charged and do not attract each other. Their negative surface charge is, however, counterbalanced by the cations in the soil solution (Mills, 2003). The pH also has a direct influence on microorganisms, mainly mediated by its effects on enzyme activities (Niemi & Vepsalainen, 2005), and thus their metabolism.

Interactions with pesticides

Organic pesticides are a versatile group of compounds that can be aromatic or aliphatic, have various functional groups and be ionic, polar or nonpolar. Like any other compound the sorption interaction with soil particles depends on the chemical properties of the organic pesticide and the properties of the soil. The strength of these interactions can vary from the relatively weak bonds of van der Waals forces (which do, however, increase in strength with increases in contact area) to the stronger bonds of covalent character (Koskinen & Harper, 1990).

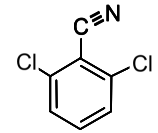
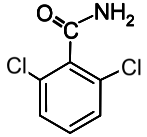
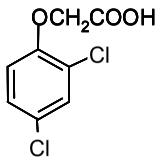
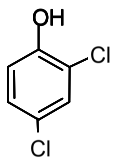
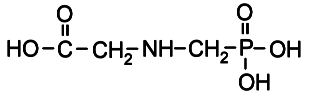
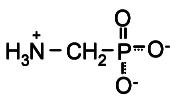
Another type of sorption interaction is the hydrophobic partitioning of nonpolar, hydrophobic compounds to organic matter (McBride, 1994). A compound in the soil may thus be influenced by several different sorption interactions with soil particles, and the potential number of sorption processes that affect it increases with increases in the compound's polarity, number of functional groups and ionisation (Koskinen & Harper, 1990). The pH controls the speciation of the compound, which can be determined from its pK_a. The species that dominates at a certain pH thereby also determines the nature of soil sorption interactions. However, since pH not only affects the speciation of the compound, but also the soil environment as a whole, effects of pH can be difficult to interpret.

Investigated pesticides

Solubility and sorption properties

The model pesticides used in the experiments (Papers I-III, table 1) range from highly hydrophobic to highly hydrophilic organic compounds. The pesticide 2,6-dichlorobenzonitrile (dichlobenil) (table 1) is a nonpolar compound that has very low solubility in water and is insensitive to low pH (EPA, 1998). The proportions of dichlobenil that sorb to sediments have been found to correlate with the organic matter contents of the sediments (Jernlås, 1990, Clausen et al., 2004), but Sheng et al. (2001) have shown that K^+-saturated smectite can sorb dichlobenil to an even higher extent than soil organic matter. The main metabolite of dichlobenil is 2,6-dichlorobenzamide (BAM) (table 1), (Beynon & Wright, 1972), which readily dissolves in water. BAM sorbs to sediments to a lesser extent than dichlobenil, however the sorption of BAM is also correlated to the organic content of the soil (Clausen et al., 2004). Sorption of the chlorophenol 2,4-DCP is strongly positively correlated with soil organic contents, and in highly alkaline environments the high cation concentrations also promote adsorption of ionized chlorophenols (Schellenberg et al., 1984). The pesticide N-phosphonomethylglycine (glyphosate) (table 1) is a polar compound and is readily dissociated in water. In soil, glyphosate is rapidly adsorbed and its adsorption is positively related to the presence of aluminium and iron oxides (Gerritse et al., 1996; Morillo et al., 2000; Gimsing and Borggaard, 2002).

Table 1. Physico-chemical properties of the pesticides dichlobenil, 2,4-D and glyphosate and their degradation products BAM, 2,4-DCP and AMPA.

	Dichlobenil	BAM	2,4-D	2,4-DCP	Glyphosate	AMPA
Structural formula						
Chemical name	2,6-dichloro-benzonitrile	2,6-dichloro-benzamide	2,4-dichloro-phenoxyacetic acid	2,4-dichloro-phenol	N-phosphonomethylglycine	Aminomethyl-phosphonic acid
Molecular weight (g mol^{-1})	172.01	190.03	221.04	163.01	169.07	110.02
Vapor pressure (mPa)	88 (20 °C) [a]	-	0.02 (25 °C) [a]	16 * 10^3 (20 °C) [b]	negligible [c]	-
Solubility (mg L^{-1})	18 (20 °C) [a]	-	20031 (pH 5, 25 °C) [a]	4600 (20 °C) [d]	12 * 10^3 [e]	-
Log K_{OW}	2.70 [a]	0.77 [f]	0.04-0.33 (pH 5) [a]	3.17 [g]	0.94 [h]	-
pK_a	-	-	2.73 [a]	7.85 [i]	< 2, 2.6, 5.6, 10.6 [j]	-
LD_{50} (rat) (mg kg^{-1})	4460 [k]	1144-2330 [l]	639-764 [a]	47 [m]	4300 [k]	-
LC_{50} (fish) (mg L^{-1})	22 (48 h) [d]	-	27-300 (96 h) [d]	5.5-8.2 (96 h) [d]	120-170 (48 h) [c]	-

LD_{50}: Oral dose resulting in 50% lethality
LC_{50}: Concentration resulting in 50% lethality
- Data not found
Reference: [a]Tomlin 2003, [b]Callahan et al. 1979, [c]EPA, 1993, [d]Verschueren 1996, [e]Shiu et al., 1990, [f]Nakagawa et al. 1992, [g]Sangster, 1993, , [h]Finizio et al., 1997, [i]Schellenberg et al., 1984, [j]Sprankle et al. 1975b, [k]Reigart & Roberts, 1999, [l]Cohr & Simonsen, 2004, [m]Sigma-Aldrich.

Environmental fate and distribution

Glyphosate and 2,4-DCP are pesticides that have been quite thoroughly studied (e.g. Haney et al., 2000, Jensen, 1996), but fewer studies have addressed the detrimental effects of dichlobenil and BAM on microorganisms in soils and water (exceptions include those published by Nikolova & Bakalivanov, 1972, Vosáhlová et al., 1997, Heinonen-Tanski, 1981). Dichlobenil is used both as an aquatic and a terrestrial broadleaf pesticide, and although it has been banned in Sweden since 1990, globally it is one of the most widely used pesticides. The major environmental concern related to dichlobenil and BAM is their dispersal to drinking water and BAM has been found in many water supply wells globally (e.g. Clausen et al., 2002, Droz et al., 2005). The dispersal of dichlobenil in soils and surface waters occurs mainly through volatilization (EPA, 1998). Though BAM has often been shown to be resistant to further degradation both in soil and culture studies (Verloop & Nimmo, 1970, Miyazaki, et al., 1975, Holtze et al., 2006), a recent study by Simonsen et al. (2006) detected rapid mineralization of BAM in a soil precontaminated with dichlobenil.

The chlorophenol 2,4-DCP can be generated as an impurity in the production of the pesticide 2,4-dichlorophenoxyacetic acid (2,4-D) (Jensen, 1996) (table 1) and is also formed as a metabolite of the pesticide (Greer et al., 1990). 2,4-DCP is a lipophilic compound that hampers catabolism by acting as an uncoupler inhibiting the oxidative phosphorylation of ATP (Terada 1990, Escher et al., 1996). The persistency of chlorophenols in soils is highly influenced by their concentration, the organic matter content of the soil, temperature and the presence of microorganisms that are able to degrade them (e.g., Edgehill, 1999, Steinle et al., 2000, Bengtsson & Carlsson, 2001, Sponza & Uluköy, 2005).

Glyphosate is a broad-spectrum pesticide and is claimed to be the most commonly used pesticide around the world in forestry and agriculture. Recent studies have shown that glyphosate can stimulate microbial activity (Haney et al., 2000, 2002, Busse et al., 2001, Araújo et al., 2003) and few studies have found any evidence that it has harmful effects on soil microorganisms (Busse et al., 2001). Glyphosate is also minimally dispersed in soil since both glyphosate and its primary metabolite in soil, aminomethylphosphonic acid (AMPA) (Rueppel et al. 1977) (table 1), strongly absorb to soil particles (Sprankle et al., 1975ab). AMPA is less susceptible to microbial degradation than glyphosate since it tends to sorb more strongly to soil surfaces (Torstensson, 1985).

Techniques

Various techniques can be used to study microbial metabolism under different environmental conditions. General techniques for measuring microbial activity in natural samples include (*inter alia*) measurements of respiration, ATP contents and the incorporation of tritiated [^{3}H]lysine or [^{3}H]thymidine into protein and DNA, respectively. In combination with various experimental setups such techniques can provide valuable information on specific growth characteristics. A common method for studies of microbial degradation is ^{14}C-labelling of the

compound considered. The respired $^{14}CO_2$ can then be used as a measure of the microbial degradation of the compound. In order to identify various organic compounds GC-MS and IR spectroscopy techniques are commonly used. An advantage with GC-MS is that the compounds can be quantified, while using IR only relative comparisons between treatments are possible. However, with IR techniques information on the interactions between the studied compound(s) and the soil surfaces can be obtained. Thus, combined analyses of microbial respiration kinetics and molecular level soil chemistry can be used to elucidate biogeochemical processes more comprehensively than either type of analysis alone.

Objectives

The overall aim of the studies underlying this thesis was to evaluate the effects of different soil properties and organic pesticides on microbial metabolism.
Specific objectives were as follows:

- To determine the effects of interactions between pH and the pesticides BAM, dichlobenil and 2,4-DCP on microbial catabolic and anabolic processes in soils (Paper I)
- To determine the effect of soil surface pH, soil surface charge density, dichlobenil and 2,4-DCP on metabolic parameters of microbial populations (Paper II)
- To determine the effect of glyphosate sorption to goethite on microbial utilization of glyphosate-P, -N, -C (Paper III)

Material and Methods

Soil sampling and soil preparation

Inoculates in all experiments originated from samples of the organic layer collected from Norway spruce (*Picea abies*) stands in either the Nyänget catchment area (64º15'N, 19º45'E, altitude 225 m a.s.l.) in Svartberget Experimental Forest, Vindeln, northern Sweden (Papers I and III) or from a site 6 km south-east of Umeå, Sweden (altitude 25 m a.s.l.) (Paper II). Within 12 hours of sampling the soil was passed through a sieve with a 5 mm mesh to remove coarse roots and plant residues, gently homogenized and then stored in polyethylene bags at -20ºC awaiting further use (Papers I-III). The pH of the organic matter-rich soil, determined either by water extraction (0.5 h, soil:solution ratio 1:4) (Paper III) or in a soil slurry (12 h, aqueous soil solution ratio 1:3) (Papers I and II), varied between 3.6-4.1, and loss on ignition (LOI) varied between 88-94% (5h, 550ºC) (Papers I-III).

Table 2. Soil surface charge densities of soil components and in a mixture of SOM (S), goethite (G) and montmorillonite (M), as well as pH calculated at surfaces of SOM (S) and goethite (G).

System		Surface charge density, q (mmol kg^{-1}) SOM[1]	Goethite[2]	Mont[3]	Mixture[4]	pH_S[5]	pH_G[6]
H	pH 3.6	-200			-200	2.6	
H+M50	pH 3.9	-250		-300	-220	3.0	
H+M100	pH 4.0	-250		-300	-230	3.1	
H+G50	pH 5.2	-500	+12		-80	3.7	7.6
H+G100	pH 5.5	-500	+12		-40	3.9	7.8
H	pH 7.0	-1000			-1000	5.0	
H+M50	pH 7.0	-1000		-300	-880	5.0	
H+M100	pH 7.0	-1000		-300	-800	5.0	
H+G50	pH 8.2	-1100	+3		-180	6.4	8.8
H+G100	pH 8.2	-1100	+3		-110	6.4	8.8

[1]Data extrapolated from Skyllberg (1996). [2]Theoretically calculated surface charge density of singly coordinated $\equiv FeOH^{-0.5}$ groups (3.6 site/nm^2) and pzc of 9.4 using the model CD-MUSIC (Hiemstra & van Riemsdijk 1996). [3]Cation-exchange capacity, CEC = 300 $mmol_c$ kg^{-1}. [4]Mass weighted surface charge density for the mixture. [5] pH at the SOM surface (pH_S) calculated using the impermeable Sphere model (Avena et al.,1999). [6]pH at the goethite surface (pH_G) calculated as $[H_G] = [H^+] \exp(-F\Psi_G/RT)$, $\Psi_G = q/C$ ($C = 0.9$ F m^{-2}). The ionic strength was estimated to be to 0.05 M in all systems, based on conductivity measurements.

No further treatments were applied to the soil used in study III before incubation. However, $CaCO_3$ or K_2CO_3 was added to half of the soil samples used in studies I and II, respectively, to increase the pH to ~7 before incubation. The soil sampled for the study in paper II was manipulated to obtain different soil surface pH and surface charge densities by mixing the organic matter-rich soil with goethite or montmorillonite in various proportions (table 2). By passing the blends through a sieve with a 5 mm mesh samples with mineral surface areas of 50 and 100 m^2 per gram of SOM were obtained. Based on measurements obtained using a conductivity meter (Jenway 4010), ionic strength was estimated to be 0.05 M for all of the soil samples. Measurements of the soil solution pH and ionic strength were used to calculate the surface pH and surface charge density (SCD) of the different soil components (table 2). Prior to incubation, the soil moisture content was adjusted to approximately 270-280% of OM to optimize conditions for microbial growth (Ilstedt et al., 2000) (Papers I and III). The soil moisture content of the soil blends in the experiments described in Paper II was adjusted to -15 kPa using suction plates to optimize conditions for microbial growth (Ilstedt et al., 2000).

Microbial respiration measurements

Microbial response variables

To assess the microbial metabolic responses to the adjustments in the soil variables, with and without pesticides, microbial kinetic parameters were estimated by acquiring and interpreting high-resolution respiration curves. The kinetic parameters (response variables) were then used to describe the effects of

the soil variables and pesticides on anabolic and catabolic components of microbial metabolism. The catabolic processes were represented by the basal respiration rate (BR, mg CO_2 h^{-1} g^{-1} OM) and substrate-induced respiration (SIR, mg CO_2 h^{-1} g^{-1} OM) (fig. 1). The BR was calculated as the average value of ≥61 hourly measurements, after the respiration rate was considered to have stabilized. SIR is an estimate of the microbial biomass in the soil (Anderson & Domsch, 1978) and was calculated as an average of the stable respiration rate after substrate addition (Nordgren et al., 1988a). The response variables representing the anabolic processes were the slope during the exponential growth phase (μ, h^{-1}) (Marstorp & Witter, 1999), the time, after substrate addition, during which the respiration rate remained constant (lag time, h) and the maximum respiration rate ($R_{max,}$ mg CO_2 h^{-1} g^{-1} OM) (fig. 1). In addition to the parameters described above, we measured the BR before and after addition of pesticides, and the resulting values were used to calculate BR_{diff} (BR_{after} - BR_{before}) (Papers I and II), the time to reach the maximum respiration rate after substrate addition (t_{max}) (Paper III) (fig. 1) and the cumulative production of CO_2 (mg CO_2 g^{-1} OM) (Paper I) during the time between the substrate (glucose, nitrogen and phosphorus) addition and the respiration rate peaking in the control samples, i.e. those to which no pesticides had been added. Cumulative CO_2 respiration integrates a number of metabolic responses that are only partly correlated with the other variables (the highest correlation was with μ; Pearson correlation r=0.66, p=0.004).

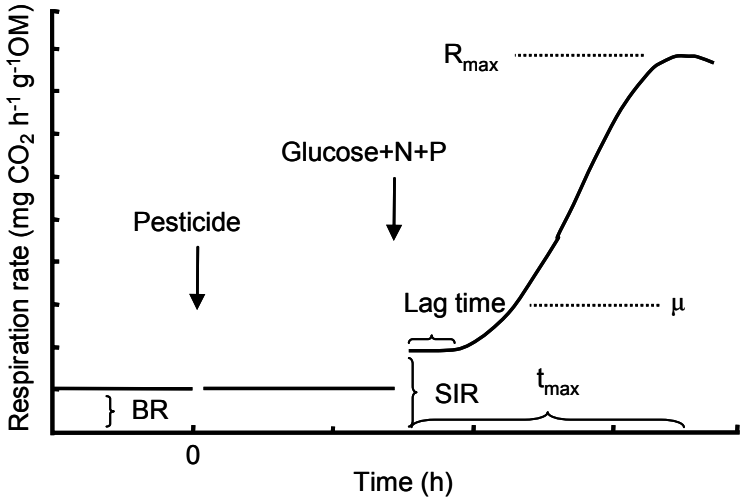


Figure 1. Typical respiration curve from the soil incubations showing descriptors of microbial catabolism (BR and SIR) and of microbial anabolism (μ) and the lag phase. BR is the basal respiration (mg CO_2 h^{-1} g^{-1} OM), and SIR (substrate-induced respiration, mg CO_2 h^{-1} g^{-1} OM) was calculated as the average rate of respiration during the period of constant respiration following the addition of glucose. The lag time (h) is the length of the period during which the respiration rate remained constant following the substrate addition and the exponential growth (μ, h^{-1}) was estimated from the natural logarithm of the respiration rate (mg CO_2 h^{-1} g^{-1} OM) and time (h). R_{max} is the maximum respiration rate (mg CO_2 h^{-1} g^{-1} OM) and t_{max} (h) is the time taken to reach R_{max}.

Incubation procedures

Briefly, the soil preparations used for the respiration kinetics analyses were first incubated without any additions, then the pesticide was added and the incubation was continued (Papers I-III). Microbial metabolism during these stages of the incubations should theoretically have been largely catabolic. To stimulate microbial growth a carbon source (glucose) and growth rate-limiting nutrients (nitrogen and phosphorus) were then added (Papers I and II). The pesticides were added either prior to addition of glucose and nitrogen (Papers I, II and III), or at the same time as the glucose, with either nitrogen or phosphorus, and both with and without goethite (Paper III only). The amount of soil used in all of the incubations corresponded to approximately one gram of dry organic matter (OM) (Papers I-III). The pesticide concentrations used in the studies described in Papers I and II were as follows: 100 (Paper I only) and 500 mg g^{-1} OM dichlobenil; 100 (Paper I only) and 500 mg g^{-1} OM BAM and 3, 10 (Paper I only) and 30 mg g^{-1} OM 2,4-DCP. In the studies described in Paper III glyphosate additions were as follows: 4.92 µmol in experiment 1; 0, 10^2, 10^3, 10^4, 10^5 µg in experiment 2; and 8.92 mg g^{-1} OM in experiment 3. During the incubations respiration kinetics were recorded hourly at 20 °C (±0.1 °C), using an automated respirometer (Respicond III) (Nordgren, 1992 and 1988). Each 250 mL plastic jar held a small container with 0.6 M potassium hydroxide solution, which reacts with respired CO_2. The decrease in conductance occurring when carbonate ions were formed from CO_2 and OH^- ions was monitored through permanently installed platinum electrodes.

Rationale for experimental conditions

For the studies on the interactions between soil properties, pesticides and microbial metabolism in soils, BAM was chosen due to the awareness of its role in contamination following releases of dichobenil and the limited knowledge of its interactions with microbial metabolism. Dichlobenil is the mother compound of BAM, and has very different properties in terms of hydrophobicity and solubility. In the experiments, due to its low solubility only some of the added dichlobenil was dissolved. The third compound in the study, 2,4-DCP, was chosen for its structural similarities with BAM and dichlobenil, all of which are di-chlorinated compounds. However, all three compounds have different functional groups, which influence their behaviour in the soil environment and their effects on microorganisms. The general toxicity of 2,4-DCP is considered to be high, while BAM and dichlobenil have moderate general toxic effects (table 1). Glyphosate was chosen for the degradation study since it has been thoroughly studied in many respects and is believed to be relatively highly degradable. The main objectives of the studies were to explore some of the mechanisms involved in the interactions between soil properties, the pesticides and microbial activity in soil samples.

One of the main focuses was on the importance of soil particle surfaces for microbial activities, molecular level chemical events, and the interactions between them. The importance of the organic material for soil processes is widely recognized. Therefore we used organic material from the O_h-horizon of a spodozol as a model system. The organic material was then mixed with mineral fractions

with differing surface charge densities. These model systems, with very high contents of organic material and clay minerals with high surface charge densities, were then used to evaluate the interactions between soil particle surfaces, pesticides and microorganisms. Due to the high content of organic material and high mineral particle surface charge density high concentrations of the substances were required.

Attenuated total reflectance-Fourier transform infra-red (ATR-FTIR) spectroscopy (Paper III)

To study the availability of glyphosate sorbed to goethite at a molecular level, ATR-FTIR spectroscopy was used (Paper III). For this purpose, small sub-samples that were visibly rich in goethite were collected from the soil-goethite incubations (experiment 3, Paper III) and were immediately applied to the diamond surface of the ATR cell of the ATR-FTIR system. This consisted of a Bruker IFS 66v/S FTIR spectrometer fitted with a deuterated triglycine sulfate (DTGS) detector, a horizontal ATR accessory and a diamond crystal as the reflection element (SensIR Technologies). The ATR cell was kept under vacuum (3-4 mbar) during acquisition of the spectra.

Statistical evaluations

The data presented in both Papers I and III were evaluated separately by two-way analysis of variance (ANOVA), and a *post hoc* Tukey's test was used to determine whether there were significant interaction effects (MINITAB 14). Data are presented as mean values with 95% confidence intervals unless otherwise stated. In Paper II, two multivariate statistical techniques were used: principal component analysis (PCA) and partial least squares projection to latent structures (PLS). PCA generates a set of axes, called principal components (PCs), oriented in multidimensional space. The first axis (PC1, the new independent variable) explains most of the variance (Wold et al., 1987). It is determined by calculating the mean, centring the data, then determining the orientation that minimises the residuals between this axis and the data points (Wold et al., 1987). The next component (PC2, calculated in the same way) is orthogonal to the first and accounts for as much of the remaining variance as possible. By projecting the data (observations) at a right angle to the PC, score values (t_i) are obtained (Wold et al., 1987). The score values is the distance to the origin and give information about the relationships among the observations. The impact of each variable on the model is described by the loadings (p_i), which is the cosine of the angle between the PC and a line connecting the variable to the origin (Wold et al., 1987). A variable with a high loading affects the component to a greater extent than a variable with a low loading. PLS has been developed from PCA and principal component regression (PCR) (Wold et al., 1987). Multiple linear regression (MLR) is used to predict the response (Y) of the PCs derived from PCA. PCR provides no information about the relationships between the X and Y matrices, but PLS attempts to maximize the covariance matrices between X and Y.

Results and Discussion

Systematic variation of soil variables affecting microbial catabolic and anabolic responses (Paper II)

Microbial metabolism can be divided into catabolic and anabolic processes. Broadly, the catabolic processes are those required to sustain life, while the main function of the anabolic processes is to build new biomass. Partly due to their contrasting key functions these processes have differing requirements in terms of carbon and nutrient source availability and are limited by the activities of various enzymes. Thus, it seems reasonable to assume that catabolic and anabolic processes differ in their responses to variations in soil parameters.

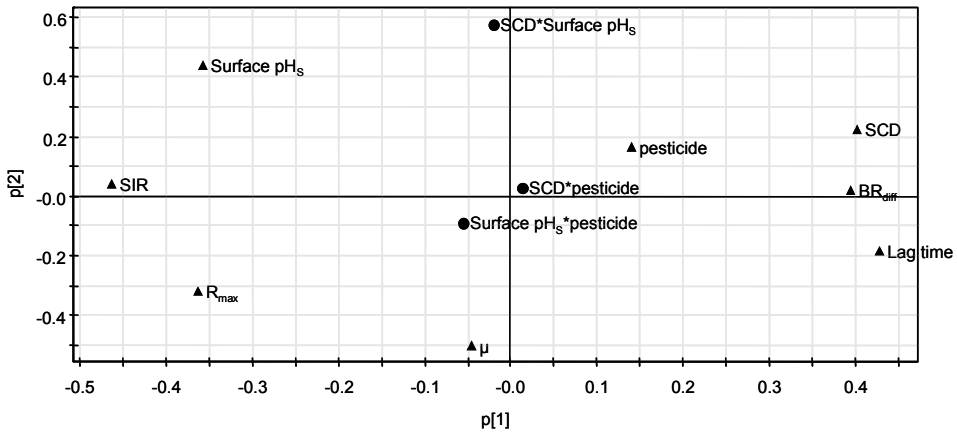


Figure 2. Loading plot, i.e. the relationships between variables, based on the principal component analysis (PCA) of the data on the microbial response variables and experimental variables (only data from samples with no pesticide or with dichlobenil included). The two components together explained 56% of the variance; PC1 33% and PC2 23%.

The most pronounced result of the PCA analysis was the separation of the catabolic response variables (BR_{diff} and SIR) and the anabolic response variable exponential growth (μ). BR_{diff} and SIR were highly correlated with each other and described only by PC1 (fig. 2), i.e. they provide similar information about the microbial response to the experimental variables. The effect of the experimental variables on μ was only correlated to PC2 and uncorrelated to BR_{diff} and SIR (fig. 2). The experimental variables surface pH (pH_S) and surface charge density (SCD) were highly correlated to PC1 and thus to the microbial response variables correlated to PC1 (SIR, BR_{diff} and lag time, and somewhat less to R_{max}) (fig. 2). The interaction term between pH_S and SCD correlated only to PC2 and was therefore, together with pH_S, most influential on the exponential growth (μ) (fig. 2). The PCA analysis revealed that the fundamental difference between catabolic and anabolic processes was reflected in the differences in their responses to variations in soil conditions.

Effects of the soil parameters on microbial response variables (Papers I and II)

Overall, increasing the pH had a significant positive effect on both the catabolic and anabolic response variables. For example: Basal respiration rate (BR) increased from 0.04 ± 0.00 (average ± 95 % confidence interval, n=24) at native pH (4.1) to 0.14 ± 0.00 at the higher pH (7.3) (Paper I), while substrate-induced respiration (SIR) was increased about two-fold (fig. 3b) (Paper I); exponential growth (μ) was increased by 45 % and lag time was reduced by ≥ 50% (fig. 3b and d) (Paper I).

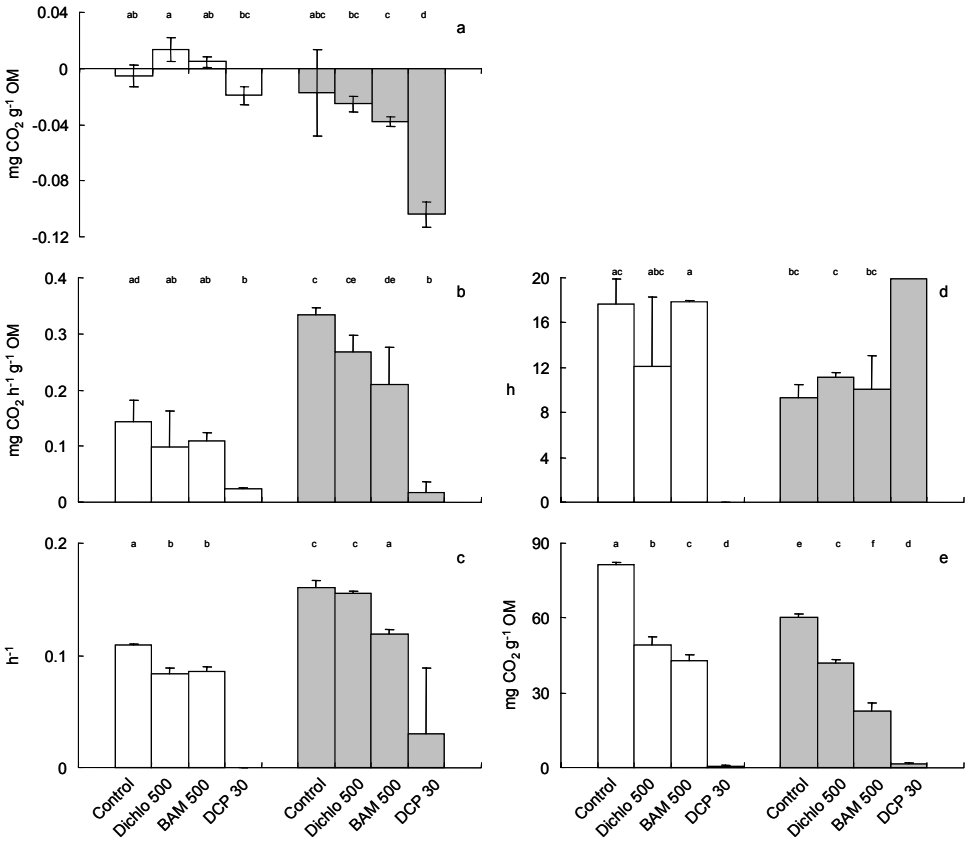


Figure 3. Effects of dichlobenil and BAM [500 mg g^{-1} OM] and 2,4-DCP [30 mg g^{-1} OM] at pH 4.1 [white] and 7.3 [grey] on the measured kinetic variables. (a) difference in respiration (BR_{diff}) rate before and after pesticide addition, (b) SIR, (c) μ, (d) lag time, and (e) cumulative CO_2 production following glucose + N + P addition, after 58 and 31 h at pH 4.1 and 7.3, respectively. Different letters above the bars indicate significant differences ($P \leq 0.05$).

The positive effects of increasing the pH on the microbial variables could be associated with many different processes in the soil. For instance, if the pH is increased the microorganisms should theoretically gain more energy from the proton gradients across their membranes, which should favour both catabolic and

anabolic responses. Another favourable effect of increases in pH is that they tend to increase the activities of many soil enzymes (Niemi & Vepsalainen, 2005). Increasing the pH also tends to make the structure of the soil humus looser, thereby increasing the availability of sorbed or bound organic compounds and cations to microorganisms (McBride, 1994). For the catabolic responses this is likely to have had a substantial effect, although the nutrient additions themselves probably had greater effects on the anabolic responses.

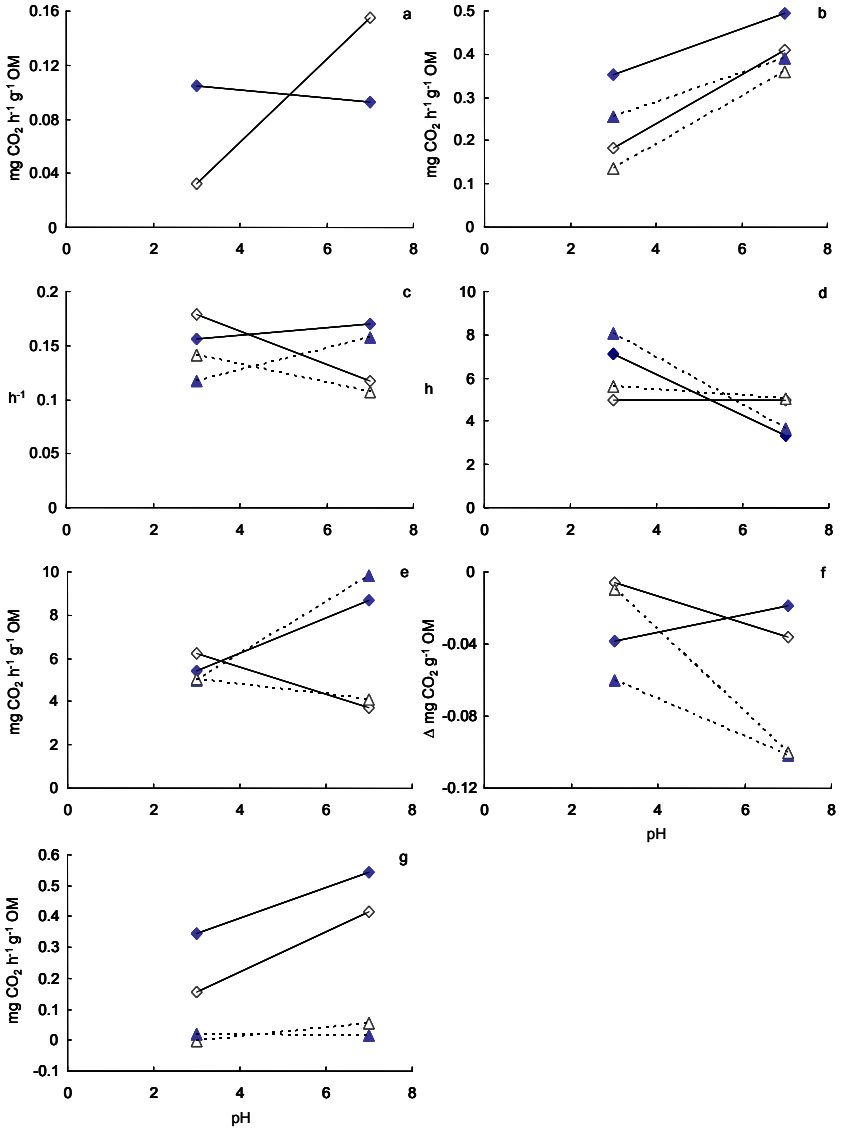


Figure 4. Effects of the significant interaction between surface pH_H and SCD on the microbial response variables. The symbols represent no pesticide addition [diamonds] and pesticide addition [triangles] at high SCD [black] and low SCD [white]: (a) BR and (with dichlobenil additions) (b) SIR, (c) μ, (d) lag time, (e) R_{max}; and (with 2,4-DCP additions) (f) BR_{diff} and (g) SIR.

Microbial metabolic processes were found to differ in their responses to changes in the soil SCD (fig. 2). Substrate-induced respiration was positively correlated with SCD independently of surface pH_S (fig. 4b), indicating that the activity of enzymes involved in substrate degradation was increased by increases in SCD. This conclusion is consistent with previous findings showing that enzyme activity can be enhanced following sorption (Ahn et al., 2002), which increases with the net charge of the surface (Bolan et al., 1999). Sorption of enzymes onto soil surfaces can increase their sustainability (Nannipieri et al., 1996), however sorption is also commonly related to reduced effective activity (Paul & Clark, 1996). Increased SCD may also have promoted sorption of the substrates (Bolan et al. 1999), thereby increasing their availability for the microorganisms.

All of the variables used to describe soil microbial activity were highly dependent on SCD and surface pH_S. Furthermore, the impact of each of these factors was highly dependent on the value of the other. Under conditions in which catabolic processes (and associated parameters like BR) dominate, access to a readily available carbon or nutrient source would not be as important for the microorganisms as it is under anabolic process-promoting conditions. Thus, the finding that increasing SCD had similar affects on BR at both investigated surface pH_S levels (fig. 4a) is consistent with theoretical expectations. At a low level of SCD, surface pH_S was highly influential on BR, possibly at least partly because the activities of exoenzymes in soil are pH-dependent (Niemi & Vepsalainen, 2005) (fig. 4a). Anabolic variables (μ, lag time and R_{max}) showed unambiguous responses to the interaction between surface charge density and surface pH_S. High SCD was more favourable at the high level of surface pH_S and the low level of SCD was more favourable at the low level of surface pH_S (fig. 4c-e). The positive anabolic responses to the interactive effects of high SCD and surface pH_S correspond to the effects of the two soil variables in isolation, as discussed above. Another factor that could have enhanced the positive anabolic response to the advantageous interactive effects of low SCD and pH_S under these conditions is the high activity of enzymes whose activities are not positively correlated with sorption (George et al., 2005) and that have low pH optima (Niemi & Vepsalainen, 2005).

Effects of the pesticides on microbial metabolism and their interactions with the soil parameters (Papers I and II)

Additions of BAM had significant negative effects on both catabolic ($p_{SIR}=0.006$) and anabolic processes ($p_{\mu}<0.001$, $p_{cumulatedCO_2}<0.001$) (table 3) (paper I). Dichlobenil additions also negatively affected microbial catabolic (fig. 4b) and anabolic processes ($p_{cumulatedCO_2}<0.001$) (fig. 3e and 4c-d) (paper I-II), but additions of 2,4-DCP had the strongest negative effect on microbial metabolism, severely affecting both catabolic and anabolic processes ($p<0.001$) (Paper I).

Table 3. Microbial respiration responses to the pesticides before and after glucose + N + P addition, the values represent average and 95% - confidence intervals.

Treatment				BR_{diff}	SIR	μ	Lag time	Cumulated CO_2 after glucose addition
Pesticide	pH	mg g^{-1} OM dw	n	Δ mg CO_2 g^{-1} OM	mg CO_2 h^{-1} g^{-1} OM	h^{-1}	h	mg CO_2 g^{-1} OM
Dichlobenil	Native	0	2	-0.01 ± 0.01	0.14 ± 0.04	0.11 ± 0.00	17.7 ± 2.2	81.4 ± 1.0 a[↓]
		100	3	0.01 ± 0.00	0.11 ± 0.05	0.09 ± 0.02	15.9 ± 3.8	44.3 ± 2.1 b [†]
		500	3	0.01 ± 0.01	0.10 ± 0.06	0.08 ± 0.01	12.1 ± 6.1	49.2 ± 3.3 b
	Raised	0	3	-0.02 ± 0.03	0.33 ± 0.01	0.16 ± 0.01	9.3 ± 1.2	60.2 ± 1.5 c
		100	3	-0.03 ± 0.00	0.29 ± 0.01	0.15 ± 0.01	10.7 ± 0.1	46.7 ± 5.8 b
		500	3	-0.03 ± 0.01	0.27 ± 0.03	0.16 ± 0.00	11.1 ± 0.5	41.7 ± 1.7 b
BAM	Native	0	2	-0.01 ± 0.01	0.14 ± 0.04	0.11 ± 0.00 a	17.7 ± 2.2	81.4 ± 1.0 a
		100	3	0.00 ± 0.00	0.11 ± 0.02	0.08 ± 0.00 b	18.9 ± 2.6	36.4 ± 0.9 b
		500	3	0.00 ± 0.00	0.11 ± 0.01	0.09 ± 0.00 b	17.9 ± 0.1	42.8 ± 2.4 c
	Raised	0	3	-0.02 ± 0.03	0.33 ± 0.01	0.16 ± 0.01 c	9.3 ± 1.2	60.2 ± 1.5 d
		100	3	-0.03 ± 0.01	0.25 ± 0.03	0.13 ± 0.01 d	11.1 ± 0.2	26.7 ± 1.5 e
		500	3	-0.04 ± 0.00	0.21 ± 0.07	0.12 ± 0.00 ad	10.1 ± 2.3	22.8 ± 2.9 e
2,4-DCP	Native	0	2	-0.01 ± 0.01 ab	0.14 ± 0.04 a	0.11 ± 0.00 a	17.7 ± 2.2	81.4 ± 1.0 a
		3	3	-0.01 ± 0.00 ab	0.16 ± 0.02 a	0.11 ± 0.00 a	19.4 ± 0.6	81.9 ± 5.8 a
		10	3	0.02 ± 0.02 a	0.05 ± 0.02 b	0.00 ± 0.00 b	-	1.7 ± 0.8 b
		30	3	-0.02 ± 0.01 ab	0.02 ± 0.00 b	0.00 ± 0.00 b	-	0.6 ± 0.2 b
	Raised	0	3	-0.02 ± 0.03 ab	0.33 ± 0.01 c	0.16 ± 0.01 ac	9.3 ± 1.2	60.2 ± 1.5 c
		3	3	-0.02 ± 0.01 ab	0.29 ± 0.05 c	0.15 ± 0.03 ac	9.3 ± 1.0	51.0 ± 10.4 c
		10	3	-0.03 ± 0.01 b	0.14 ± 0.04 a	0.19 ± 0.01 c	4.0 ± 0.5	63.8 ± 10.3 c
		30	3	-0.10 ± 0.01 c	0.02 ± 0.02 b	0.03 ± 0.06 b [‡]	19.8 ± 0.0 [‡]	1.3 ± 0.6 b [†]

[†] - Missing value, n-1
[‡] - Exponential growth occurred in only one replicate
[↓] - Different letters denote significant differences ($P \leq 0.05$) between interactions.

Initially, toxic effects of 2,4-DCP additions were more pronounced on the catabolic processes at the raised pH than in incubations at the native pH (table 3, fig. 3a). However, after the addition of substrate (glucose) and nutrients (nitrogen and phosphate), increased pH seemed to reduce the toxic effect of the additions of 2,4-DCP (table 3, fig. 3b-e). A probable contributory factor to the stronger effect of 2,4-DCP at the lower pH is that 2,4-DCP most readily enters cells in its undissociated form, and at the raised pH (7.3) approximately 20 % of the added 2,4-DCP (pK_a 7.85) should theoretically have been in its anionic form. Although 2,4-DCP anions are also capable of passing through the microbial membrane, the proportions entering cells at pH 7.0 have been found to be minor (1%) and insufficient to cause measurable uncoupling activity (Escher et al., 1996). The amount of the anionic form available to enter the microbial cells is further reduced by sorption, which is strongly correlated to the soil organic content (Schellenberg et al., 1984). This also applies to the undissociated form, but the sorption kinetics and equilibrium states of the two forms differ, and are strongly influenced by various environmental factors. These theoretical considerations and empirical observations indicate that, in the system studied, at the raised pH some of the 2,4-DCP was in its dissociated form, which may explain at least some of its lower toxicity at this pH.

In contrast to 2,4-DCP, dichlobenil and BAM are stable in the pH range studied (EPA, 1998). Thus, the impact of pH on microbial responses to dichlobenil and BAM can be attributed to the effects on soil organic matter properties on the pesticides *per se* and/or their direct or indirect effects on microorganisms. In addition, dichlobenil had more pronounced effects on microbial metabolism (cumulative CO_2 respiration) at native pH (table 3, fig. 3e). Due to its hydrophobicity, dichlobenil partitions more strongly to the surface of the soil particles than into the soil solution. At native pH the soil organic matter had a lower net surface charge and was more hydrophobic than at the raised pH. This may have contributed to the difference in inhibitory effects of dichlobenil on the microorganisms at low and high pH. In contrast, BAM affected microbial metabolic processes most strongly at the raised pH (table 3). The negative effect of BAM on microbial metabolism indicated that the amide interacted with the microorganisms and inhibited the synthesis of new cells. Raising the pH of the soil solution increases the sorption parameters of the soil organic matter by increasing the negative charge density of the soil surface (Bolan et al., 1999). Increasing the soil surface charge density slightly positively affects the generally quite weak surface sorption of BAM (Clausen et al., 2004), which may explain the increased negative effect of BAM on microbial metabolism at the higher pH.

In accordance with the theoretical considerations outlined in Paper I, the negative effects of dichlobenil additions on the catabolic response represented by SIR were also reduced at the high level of SCD (fig. 4b). The effects of the interaction between SCD and pH_S on the anabolic responses to the presence of dichlobenil followed the pattern observed in the absence of pesticide (fig. 4c-e). However, the negative effect of the dichlobenil additions was weaker when SCD and pH_S were high, further supporting the theoretical considerations discussed in Paper I.

A low level of SCD in combination with low pH_S eliminated the toxic effect of 2,4-DCP additions on basal respiration (BR_{diff}) (fig. 4f). These results are in agreement with the findings presented in Paper I, and might have been due to low microbial metabolic activity under these conditions. However, at the raised pH_S, the SCD seemed to have little influence (fig. 4f). Thus, the adverse uncoupling activity of 2,4-DCP appears to exceed the stimulatory effects of possible increases in catabolic enzyme activity under these soil conditions.

Effect of sorption on the microbial degradation of glyphosate (Paper III)

Glyphosate is an intensively studied biodegradable pesticide that can be utilized by microorganisms as a carbon (C) substrate (Haney et al., 2000, Busse et al., 2001) and as a source of both nitrogen (N) (Haney et al., 2000) and phosphorous (P) (Dick & Quinn, 1995, Krzyśko-Lupicka & Orlik, 1997). When sorbed to goethite we hypothesized that glyphosate bioavailability would be reduced. However, in the respiration experiments where the microbial availability of glyphosate-P and glyphosate-N with and without goethite was tested, no evidence for utilization of N or P derived from glyphosate was obtained (fig. 5a-d). Furthermore, the microbial anabolic response was inhibited by the glyphosate additions irrespective of goethite additions. However, sorption of glyphosate to goethite reduced the harmful effects of glyphosate (fig. 5b and d). Similar results were obtained in the respiration experiments with glyphosate as a carbon source; glyphosate inhibited both catabolic and anabolic processes, however the negative effect was reduced by the presence of goethite. Nevertheless, analysis with ATR-FTIR spectroscopy confirmed that the microorganisms could utilize glyphosate sorbed to goethite as a source of carbon through de-carboxylation (fig. 6b). The decrease in BR observed with higher glyphosate additions suggests that energy generation from the breakdown of organic substrates is inhibited by glyphosate. It also had a major impact on microbial growth, indicating that glyphosate, or its metabolites, negatively affected anabolic activity. This effect remained at higher glyphosate doses even after prolonged incubation (Paper III). More detailed ATR-FTIR spectroscopy analysis of the phosphonate region revealed that sorbed glyphosate appeared to be further oxidized to orthophosphate, which at least partly remained as surface complexes on the goethite (fig. 7g).

In the experiments with glyphosate as a carbon source the highest glyphosate addition (10^5 μg g^{-1} OM) could have induced a 2-fold increase in the respiration rate, which would have been easily detectable. This, however, was not the case, and contrasts with the results presented by Busse et al. (2001), where increased CO_2 respiration rates were detected within a similar time period using forest humus soils and glyphosate additions similar to those tested here. Previous laboratory studies have found that glyphosate has harmful effects on microorganisms (Krzyśko-Lupicka & Orlik, 1997, Christy et al., 1981, Quinn et al., 1988). Our results are in agreement with these findings, indicating that glyphosate has negative effects on the microbial anabolic processes. These results,

however, conflict with those of many other studies indicating that glyphosate has a positive effect on microbial growth (e.g. Busse et al., 2001).

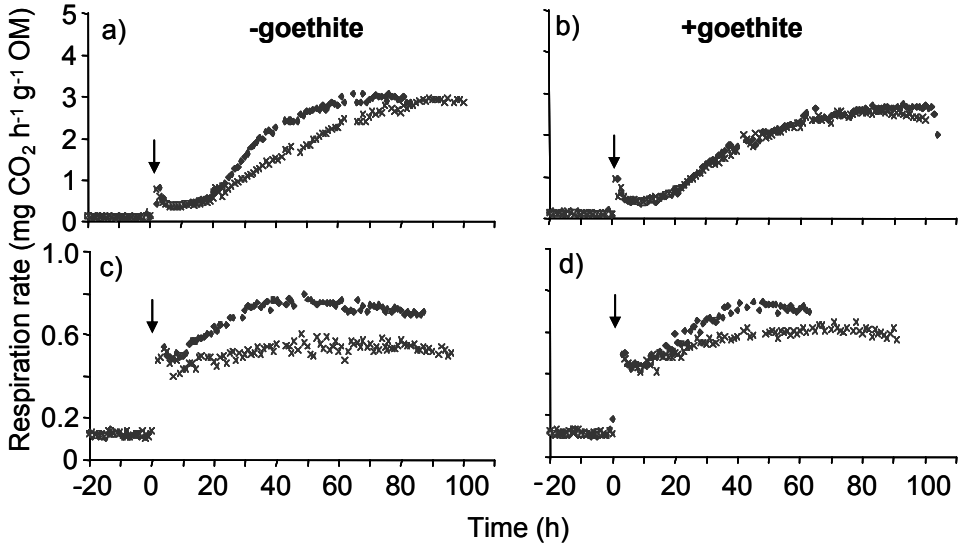


Figure 5. Microbial respiration responses to additions of glyphosate in combination with (a) glucose and N without goethite; (b) glucose and N with goethite; (c) glucose and P without goethite; and (d) glucose. Diamonds denote controls without glyphosate and the arrow denotes the time of addition.

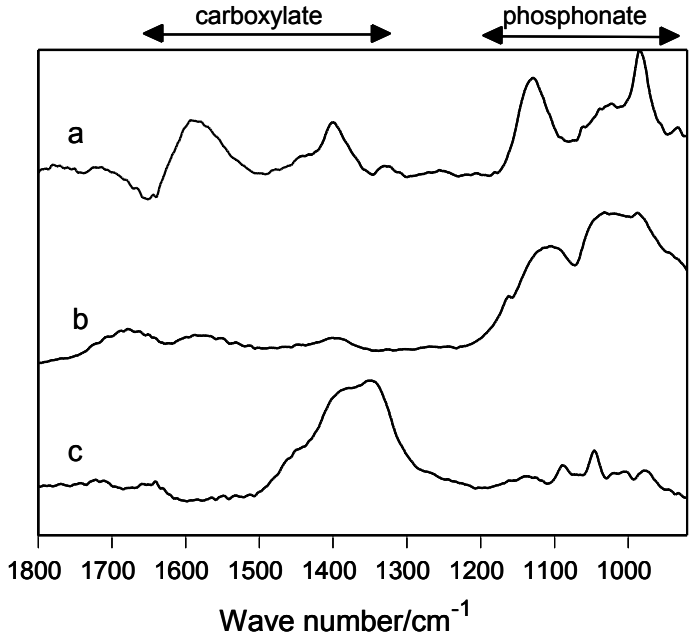


Figure 6. ATR-FTIR spectra of (a) glyphosate on goethite at pH 4.6, (b) glyphosate on goethite added to soil, (c) glyphosate on goethite added to with soil + glucose and N.

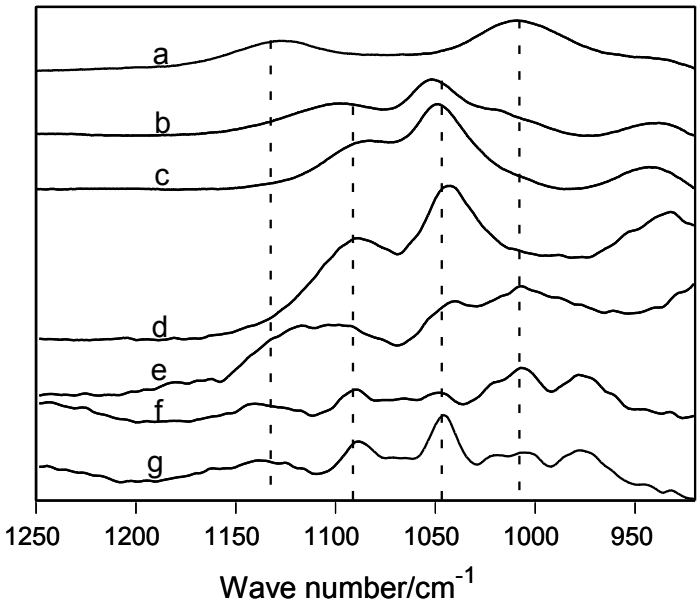


Figure 7. ATR-FTIR spectra of (a) orthophosphate on goethite at pH 2.9, (b) orthophosphate on goethite at pH 6.3, (c) orthophosphate on goethite at pH 9.8, (d) suspension used for the soil experiments containing orthophosphate on goethite at pH 4.6, (e) orthophosphate on goethite added to soil, (f) orthophosphate on goethite added to soil + glucose and N, (g) glyphosate on goethite added to soil + glucose and N.

Implications of the results and future research priorities

No other group of organisms is as essential for all of the other organisms in an ecosystem as the microorganisms. Their versatility and ability to adapt to changes in the environment is the key to their importance and success. The main implication of the results from these studies on microorganisms and their interactions with soil properties is that various soil parameters have differing effects on catabolic and anabolic microbial processes; one set of soil parameters may be more beneficial for catabolic processes while another is more beneficial for anabolic processes.

This raises questions about the optimal conditions for the microorganisms. In most soils growth is slow and maintaining the existing population should be the key objective in attempts to promote soil vitality. A key issue to address, therefore, is whether soil conditions favouring catabolic processes or conditions favouring the growth of microorganisms are most beneficial for promoting the survival and desirable functions of the microbial population.

Generally, the microbial metabolic responses in soils with contrasting properties followed the same patterns in both the presence and absence of the tested pesticides, with the exception of 2,4-DCP. However, the results for all pesticides indicate that in soil conditions that are most favourable for microbial metabolism the pesticides have the weakest negative effects. These results were obtained in

tests with a soil microbial population originating from soil that was rich in organic matter and had a naturally acidic pH. Therefore, further important issues to explore are whether soil microorganisms originating from other types of soil respond in a similar way, and whether the degradation or transformation of pesticides is more strongly promoted by their retention in organic matter-rich soils with a highly active microbial community than in other types of soil.

Sorption of a compound to a soil particle is often considered to strongly limit its microbial availability. However, these studies indicate that although sorption can delay the degradation of a compound, it also reduces the negative effects of glyphosate on microbial metabolism. Moreover, the results imply that even when sorbed to goethite, glyphosate can be fully mineralized by the microorganisms. Since goethite is a common mineral in many soils these findings should be applicable to various field conditions. In further attempts to explain biogeochemical processes combining analyses of microbial respiration kinetics with molecular level processes will be of great significance.

Major conclusions

General conclusions

- The general view of the relationship between pH and soil microbial processes is that a close to neutral pH is most beneficial for microbial activities. However, the studies this thesis is based upon clearly demonstrate that the effect of pH on soil microbial processes is highly variable and a lower pH, even in the acid range, may be favourable.

- The effect of pH on the soil microbial metabolism is highly dependent on the surface properties of the inorganic and organic fractions of the soil, as exemplified by the surface charge densities.

- The pH and surface charge properties of soils have differing effects on different components of microbial metabolism.

- The interactions between microbial metabolism and pesticides are highly dependent on both the pH and soil particle surface charge density along with interactions between the two.

More detailed conclusions are:

- Soil surface charge density (SCD) and SOM surface pH (pH_S) interactively affect microbial metabolism; at a high level of SCD an increase in pH_S is favourable and at a low level of SCD a decrease in pH_S is most beneficial.

- A low level of SCD in combination with low pH_S eliminates the toxic effect of 2,4-DCP additions on basal respiration (BR_{diff}). However,

increasing pH_S in the presence of 2,4-DCP severely affected basal respiration, independently of the SCD.

- Raising the pH reduced the toxic effects of 2,4-DCP on microbial anabolic processes.

- High levels of SCD and surface pH_S reduced the negative effects of dichlobenil on microbial metabolism.

- BAM had a greater negative effect on the microbial anabolic processes at neutral than at acid pH

- Dichlobenil had a greater negative effect on the microbial anabolic processes at acid than at neutral pH

- Glyphosate sorbed onto goethite is a microbially available source of C, N and possibly P.

Literature cited

Ahn, M.-Y., Dec, J., Kim, J.-E., Bollag, J.-M., 2002. Treatment of 2,4-dichlorophenol polluted soil with free and immobilized laccase. Journal of Environmental Quality 31, 1509–1515.

Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology & Biochemistry 10, 215-221.

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

Avena J.A, Koopal, L.K., van Riemsdijk, W.H., 1999. Proton binding to humic acids: Electrostatic and intrinsic interactions. Journal of Colloid and Interface Science 217, 37-48.

Barja, B. C.; Alfonso, M. D. Aminomethylphosphonic acid and glyphosate adsorption onto goethite: a comparative study. Environ. Sci. Technol. 2005, 39, 585-592.

Bengtsson, B., Carlsson, C., 2001. Degradation of dissolved and sorbed 2,4-dichlorophenol in soil columns by suspended and sorbed bacteria. Biodegradation 12, 419-432.

Beynon, K.I., Wright, A.N., 1972. The fates of the herbicides chlorthiamide and dichlobenil in relation to residues in crops, soils and animals. Residue Reviews 43, 23-53.

Bolan, N.S., Naidu, R., Syers, J.K., Tillman,R.W., 1999. Surface charge and solute interactions in soils. Advances in Agronomy 67, 87-140.

Bollag, J.-M., Liu, S.-Y., 1990. Biological transformation processes of pesticides. In: Cheng, H.H. (Ed.), Pesticides in soil environment: Processes, Impacts and Modeling. SSSA Book Series no. 2, Soil Science Society of America Book, Inc. Madison, WI., 169-211.

Busse, M.D., Ratcliff, A.W., Shestak, C.J., Powers, R.F., 2001. Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biology & Biochemistry 33, 1777-1789.

Callahan, M.A, Slimak, M.W., Gabel, N.W., May, I.P., Fowler, C.F., Freed, J.R., Jennings, P., Durfee, R.L.,Whitemore, F.C., Maestri, B., Mabey, W.R., Holt, B.R., Gould, C., 1979. Water-related environmental fate of 129 priority pollutants. EPA-440-4-79-029a,b. Versar Inc., Springfield, Virginia.

Cerniglia, C.E., Sutherland, J.B., Crow, S.A., 1992.Fungal metabolism of aromatic hydrocarbons. In: Winkelmann, G. (Ed.), Microbial degradation of natural products. VCH Verlagsgesellschaft mBH, Weinhem, pp. 193-217.

Christy, S.L., Karlander, E.P., Parochetti, J.V., 1981. Effects of glyphosate on the growth rate of *Chlorella*. Weed Science 29, 5-7.

Clausen, L., Arildskou, N. P., Larsen, F., 2002. Nedbrydning og sorption af dichlobenil og BAM. In Pesticides and point sources. Meeting at the Academy of Technical Sciences, January 31th 2002, pp. 55-65. (In Danish)

Clausen, L., Larsen, F., Albrechtsen, H.-J., 2004. Sorption of the herbicide dichlobenil and the metabolite 2,6-dichlorobenzamide on soils and aquifer sediments. Environmental Science & Technology 38, 4510-4518.

Cohr, K.-H., Simonsen, F.A., 2004. Evaluation of health hazards by exposure to BAM (2,6-dichlorobenzamide) and risk characterisation of drinking water exposure. Danish Toxicology Centre, The Danish EPA, Copenhagen, Denmark.

Dick, R. E.; Quinn, J. P. Glyphosate-degrading isolates from environmental samples: occurrence and pathways of degradation. Applied Microbiology & Biotechnology 1995, 43, 545-550.

Droz, C., Kolbener, P., Hunziker, H., 2005. Herbizide und Dichlorbenzamid in Grund- und Quellwasser im Kanton St. Gallen. Mitteilungen aus Lebensmitteluntersuchung und Hygiene 96, 118-126.(In German)

Edgehill, R.U., 1999. Bioremediation by inoculation with microorganisms. In: Adriano, D.C., Bollag, J.-M., Frankenberger, W.T., Jr., Sims, R.C. (Eds.), Bioremediation of Contaminated Soils, Agronomy no. 37, American Society of Agronomy Inc., Madison, WI., 820 pp.

EPA (US Environmental Protection Agency), 1993. Registration Eligibility Decision (RED): Glyphosate. EPA-738-R-93-014.
EPA (US Environmental Protection Agency), 1998. Registration Eligibility Decision (RED): Dichlobenil. EPA-738-R-98-003.
Escher, B.I., Snozzi, M., Schwarzenbach, R.P., 1996. Uptake, speciation and uncoupling activity of substituted phenols in energy transducing membranes. Environmental Science & Technology 30, 3071-3079.
Finizio, A., Vighi, M., Sandroni D., 1997. Determination of n-octanol/water partition coefficient (Kow) of pesticide critical review and comparison of methods. Chemosphere 34, 131-161.
George, T.S., Richardson, A.E., Simpson, R.J., 2005. Behaviour of plant-derived extracellular phytase upon addition to soil. Soil Biology & Biochemistry 37, 977-988.
Gerritse, R.G., Beltran, J., Hernandez, F., 1996. Adsorption of atrazine, simazine, and glyphosate in soils of the Gnangara Mound, Western Australia. Australien Journal of Soil Research 34, 599-607.
Gimsing, A.L.; Borggaard, O.K., 2002. Effect of phosphate on the adsorption of glyphosate on soils, clay minerals and oxides. International Journal of Environmental Analytical Chemistry 82, 545-552.
Greer, C.W., Hawari, J., Samson, R., 1990. Influence of environmental factors on 2,4-dichlorophenoxyacetic acid degradation by *Pseudomonas cepacia* isolated from peat. Archives of Microbiology 154, 317-322.
Haney, R.L., Senseman, S.A., Hons, F. M., Zuberer, D.A., 2000. Effect of glyphosate on soil microbial activity and biomass. Weed Science 48, 89-93.
Haney, R.L., Senseman, S.A., Hons, F. M., 2002. Effect of roundup ultra on microbial activity and biomass from selected soils. Journal of Environnmental Quality 31, 730-735.
Heinonen-Tanski, H., 1981. The interaction of microorganisms and the herbicides chlorthiamide and dichlobenil. Journal of the Scientific Agricultural Society of Finland 43, 341-390.
Holtze, M.S., Sørensen, J., Hansen, H.C.B., Aamand, J., 2006. Transformation of the herbicide 2,6-dichlorobenzonitrile to the persistent metabolite 2,6-dichlorobenzamide (BAM) by soil bacteria known to harbour nitrile hydrates and nitrilase. Biodegradation 17, 503-510.
Ilstedt, U., Nordgren, A., Malmer, A., 2000. Optimum soil water for soil respiration before and after amendment with glucose in humid tropical acrisols and boreal mor layer. Soil Biology & Biochemistry 32, 1591-1599.
Jensen, J., 1996. Chlorophenols in the terrestrial environment. Reviews of Environmental Contamination & Toxicology 146, 25-51.
Jernlås, R., 1990. Mobility in sandy soils of four pesticides with different water solubility. Acta Agriculturae Scandinavica 40, 325-340.
Koskinen, W.C., Harper, S.S, 1990. The retention process: Mechanisms. In: Cheng, H.H. (Ed.), Pesticides in soil environment: Processes, Impacts and Modeling. SSSA Book Series no. 2, Soil Science Society of America Book, Inc. Madison, WI., 51-77.
Krzyśko-Lupicka, T., Orlik, A., 1997. The use of glyphosate as the sole source of phosphorus or carbon for the selection of soil-borne fungal strains capable to degrade this herbicide. Chemosphere 34, 2601-2605.
Marstorp, H., Witter, E., 1999. Extractable dsDNA and product formation as measures of microbial growth in soil upon substrate addition. Soil Biology & Biochemistry 31, 1443-1453.
McBride, M.B., 1994. Environmental Chemistry of Soils. Oxford University Press, New York, 375 pp.
Mills, A.L., 2003. Keeping in touch: Microbial life on soil particle surfaces. Advances in Agronomy 78, 1-43.
Miyazaki, S., Sikka, H.C., Lynch, R.S., 1975. Metabolism of dichlobenil by microorganisms in aquatic environment. Journal of Agricultural & Food Chemistry 23, 365-368.
Morillo, E.; Undabeytia, T.; Maqueda, C.; Ramos, A. Glyphosate adsorption on soils of different characteristics. Influence of copper addition. Chemosphere 2000 40, 103-107.

Nakagawa, Y., Izumi, K., Oikawa, N., Sotomatsu, t., Shigemura, M., Fujita, t., 1992. Analysis and prediction of hydrophobicity parameters of substituted acetanilides, benzamides and related aromatic compounds. Environmental Toxicology & Chemistry 11, 901-916.

Nannipieri, P., Sequi, P., Fusi, P., 1996. Humus and enzymatic activity. In: Piccolo, A. (Ed.), Humic substances in terrestrial ecosystems. Elsevier, Amsterdam, 293-328.

Niemi, R.M., Vepsalainen, M., 2005. Stability of the fluorogenic enzyme substrates and pH optima of enzyme activities in different Finnish soils. Journal of Microbiological Methods 60, 195-205.

Nikolova, G., Bakalivanov, D., 1972. Microbiological activity and breakdown of "Casoron" herbicide in the soil. Proceedings of the Symposium on Soil Microbiology, Budapest, 1970. Symposia Biologica Hungarica 11, 385-389.

Nordgren, A., Bååth, E., Söderström, B., 1988a. Evaluation of soil respiration characteristics to assess heavy metal effects on soil microorganisms using glutamic acid as a substrate. Soil Biology & Biochemistry 20, 949-954.

Nordgren, A., 1988b. Apparatus for the continuous, long-term monitoring of soil respiration rate in large numbers of samples. Soil Biology & Biochemistry 20, 955-957.

Nordgren, A., 1992. A method for determining microbially available N and P in an organic soil. Biology & Fertility of Soils 13, 195-199.

Quinn, J.P., Peden, J.M.M., Dick, R.E., 1988. Glyphosate tolerance and utilization by the microflora of soils treated with the herbicide. Applied Microbiology & Biotechnology 29, 511-516.

Paul, E.A., Clark, F.E., 1996. Soil microbiology and biochemistry, 2nd ed. Academic Press, Elsevier Science, San Diego, CA, 340 pp.

Reigart, J.R., Roberts, J.R., 1999. Recognition and management of pesticide poisonings, 5th ed. EPA (US Environmental Protection Agency). Available at: http://www.epa.gov/pesticides/safety/healthcare

Rueppel, M.L., Brigthwell, B.B., Schaefer, J., Marvel, J.T., 1977. Metabolism and degradation of glyphosate in soil and water. Journal of Agricultural & Food Chemistry 25, 517-528.

Sangster, J., 1993. LOGKOW computer databank, Sangster Research Labs., Montreal, Canada.

Schellenberg, K., Leuenberger, C., Schwarzenbach, R.P., 1984. Sorption of chlorinated phenols by natural sediments and aquifer materials. Environmental Science & Technology 18, 652-657.

Sheals, J., Sjöberg, S., Persson, P., 2002. Adsorption of glyphosate on goethite: molecular characterization of surface complexes. Environmental Science & Technology, 36, 3090-3095.

Sheng, G., Johnston, C.T., Teppen, B.J., Boyd, S.A., 2001. Potential contributions of smectite clays and organic matter to pesticide retention in soils. Journal of Agricultural & Food Chemistry 49, 2899-2907.

Shiu, W.Y., Ma, K.C., Mackay, D., Seiber, J.N., Wauchope, R.D., 1990. Reviews of environmental contamination and toxicology, vol. 116, Springer-Verlag, NY, 199 pp.

Simonsen, A., Holtze, M.S, Sørensen, S.R., Sørensen, S.J., Aamand, J., 2006. Mineralisation of 2,6-dichlorobenzamide (BAM) in dichlobenil-exposed soils and isolation of a BAM-mineralising *Aminobacter* sp. Environmental Pollution 144, 289-295.

Skyllberg, U., 1996. Small scale pH buffering in organic horizons of two boreal coniferous forests stands. Plant & Soil 179, 99-107.

Smith, J.L., Papendick, R.I., Bezdicek, D.F., Lynch, J.M., 1993. Soil organic matter dynamics and crop residue management. In: Metting, F.B. (Ed.), Soil Microbial Ecology: Applications in Agricultural and Environmental Management, 65-94.

Sponza, D.T., Uluköy, A., 2005. Treatment of 2,4-dichlorophenol (DCP) in a sequential anaerobic (upflow anaerobic sludge blanket) aerobic (completely stirred tank) reactor system. Process Biochemistry 40, 3419-3428.

Sposito, G., 1989. The Chemistry of Soils. Oxford University Press, New York, 277 pp.

Sprankle, P., Meggitt, W.F., Penner, D., 1975a. Rapid inactivation of glyphosate in the soil. Weed Science, vol. 23, (3) 224-228.

Sprankle, P., Meggitt, W.F., Penner, D., 1975b. Adsorption, mobility, and microbial degradation of glyphosate in the soil. Weed Science, vol. 23, (3) 229-234.
Stark, W.H., Stadler, J., McCoy, E., 1938. Some factors affecting the bacterial population of freshwater lakes. Journal of Bacteriology 36, 653-654.
Steinle, P., Thalmann, P., Höhener, P., Hanselmann, K.W., Stucki, G., 2000. Effect of environmental factors on the degradation of 2,6-dichlorophenol in soil. Environmental Science & Technology 34, 771-775.
Terada, H., 1990. Uncouplers of oxidative phosphorylation. Environmental Health Perspectives 87, 213-218.
Tomlin, C. (ed.). 2003. The pesticide manual. 13th Ed. British Crop Protection Council, Alton, UK, xxvi +1344 pp.
Torstensson, L., 1985. Behavior of glyphosate in soils and its degradation. In: Grossbard, E., Atkinson, D., (Eds.), The Herbicide Glyphosate. Butterworths, London, 137-150.
Verloop, A., Nimmo, W.B., 1970. Metabolism of dichlobenil in sandy soil. Weed Research 10, 65-70.
Verschueren, K., 1996. Handbook of environmental data on organic chemicals. 3rd ed., Van Nostrand Reinhold Co., New York, NY., 2064 pp.
Vosáhlová, J., Pavlů, L., Vosáhlo, J., Brenner, V., 1997. Degradation of bromoxynil, ioxynil, dichlobenil and their mixtures by *Agrobacterium radiobacter* 8/4. Pesticide Science 49, 303-306.

Acknowledgements

Projeketet finanserades av EUs strukturfonder Mål 1 genom
Marksaneringscentrum Norr (MCN).

Först vill jag tacka min handledare Mats Nilsson, du har varit ovärderlig under min tid här som doktorand, tack för att du har alltid funnit tillhands när jag har haft frågor och för att du alltid kommit med nya (och gamla, jag kan dem utantill ;]) infallsvinklar på hur hinder ska forceras och gränser sprängas!

Mina biträdande handledare: Ulf Skyllberg, tack för all peppning och för rådfrågning om forskning och pippisar, skådningen i Sydafrika var en höjdare! Anders Nordgren, tack för all hjälp med respirometern och alla intressanta pratstunder och för att du gav mig möjlighet att doktorera!

Erik Andersson, resorna till Lund i samband med ex-jobbet och samarbetet med dig där inspirerade mig att jobba vidare med forskning, den första ”sniffen” på forskarvärlden kunde inte blivit bättre! Serendipity – en lycklig slump att just vi träffades!

Reiner Giesler och Per Persson, tack för gott samarbete med artiklar. Vad hände med Whiskey’n?

Tack alla forskningskollegor inom MCN för nyttiga och trevliga möten och studiebesök!

Alla medarbetare på institutionen för Skogsekologi, tack för att ni fått mig att trivas alldeles förträffligt, jag diggar er alla starkt! Ett särskilt tack måste dock gå till alla doktorander, gamla och nya, vi har gjort så mycket roligt tillsammans och jag kommer att sakna er alla!

Livet utanför universitetet vore inte alls lika roligt och spännande utan mina beach och volleybollvänner, det är kul att kämpa och svettas i grupp! Mina ridkompisar, tack för alla skratt och fester där det mesta avhandlats! Tack alla vänner här i Umeå som fungerat som säkerhetsventiler när tankarna i huvudet krockat och oaser där jag har kunnat koppla bort allt som har med forskning att göra under långpass i ur o skur eller över ett tufft parti bridge eller Settlers, näe, jag är inte alls någon tävlingsmänniska…

Vänner långt bort, men ändå alltid nära, tack för att ni alltid finns när det behövs!

Avkoppling och middager med min ”ume/vägsele släkt” har varit ett mycket välkommet och välbehövligt inslag, tack för all omtanke!

Min familj, hemma bra, men hemhemma bäst! Alltid lika härligt att få åka hem till Falun eller till Farmor i Askersund, bättre sätt att ladda batterierna på finns inte!

Utan Skype i tre dagar försmäktar jag i denna värld! Pär, tack för allt stöd via cyber-jyyymden, du är helt fantastisk!!