Biodegradation Activities in Coniferous Forest Soils and Freshwater Sediments

Mika A. Kähkönen

Department of Applied Chemistry and Microbiology
Division of Microbiology
University of Helsinki
Finland

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Supervisor: Professor Mirja Salkinoja-Salonen
Department of Applied Chemistry and Microbiology
University of Helsinki, Finland

Reviewers: Docent Aino Smolander
The Finnish Forest Research Institute
Vantaa Research Centre, Finland

Docent Aino Smolander
The Finnish Forest Research Institute
Vantaa Research Centre, Finland

Opponent: Professor Rauni Strömmer
Department of Ecological and Environmental Sciences
University of Helsinki, Lahti, Finland

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Front cover: Podzol soil profile in Hyytiälä pine forest. Photograph: Hannu Ilvesniemi (Helsinki University, Dept. of Forest Ecology)

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LIST OF ORIGINAL PAPERS

This thesis is based on the following papers, referred to in the text by their Roman numerals. *Additionally, some unpublished results are presented.*

I **Mika A. Kähkönen**, Kimmo P. Suominen, Pentti K.G. Manninen and Mirja S. Salkinoja-Salonen 1998. 100 years of sediment accumulation history of organic halogens and heavy metals in recipient and nonrecipient lakes of pulping industry in Finland. Environmental Science & Technology 32 (12), 1741-1746.

II **Mika A. Kähkönen**, Mikko Liukkonen, Christoph Wittmann, Kimmo P. Suominen and Mirja S. Salkinoja-Salonen 1999. Integrative assessment of sediment quality history in pulp mill recipient area in Finland. Water Science and Technology Vol. 40, 139-146.

III **Mika A. Kähkönen**, Christoph Wittmann, Hannu Ilvesniemi, Carl J. Westman and Mirja S. Salkinoja-Salonen 2002. Mineralization of detritus and oxidation of methane in acid boreal coniferous forest soils: Seasonal and vertical distribution and effects of clear-cut. Soil Biology & Biochemistry 34, 1191-1200.

IV **Mika A. Kähkönen**, Christoph Wittmann, Jukka Kurola, Hannu Ilvesniemi and Mirja S. Salkinoja-Salonen 2001. Microbial activity of boreal forest soil in a cold climate. Boreal Environmental Research, 6, 19-28.

V Christoph Wittmann, **Mika A. Kähkönen**, Hannu Ilvesniemi, Jukka Kurola and Mirja S. Salkinoja-Salonen 2002. Activities of hydrolytic enzymes involved in the biochemical cycles of carbon, nitrogen, sulphur and phosphorus in podsolized forest soils over nine seasons. Submitted to Soil Biology & Biochemistry.

THE AUTHOR'S CONTRIBUTION:

Paper I
Mika A. Kähkönen wrote the paper and is the corresponding author. He interpreted main part of the results. He mainly planned and performed heavy metal analysis and part of carbon analysis.

Paper II
Mika A. Kähkönen wrote the paper, is the corresponding author and interpreted the results. He mainly planned and performed heavy metal analysis. He developed and performed further calculations from the experimental results in this paper.

Paper III
Mika A. Kähkönen wrote the paper, is the corresponding author and interpreted the results. He planned and performed all experimental work, except for the measurements of air and soil temperature, methane concentration in soil air, and ammonium and nitrate content in soil water. He excluded a part of the analyses on the influence of nitrogen containing salts on methane oxidation and the kinetics of methane oxidation. Mika A. Kähkönen performed main part of the field work.

Paper IV
Mika A. Kähkönen wrote the paper and interpreted the results together with his supervisor Prof. Mirja Salkinoja-Salonen. He planned and performed all experimental work, except the mineralization experiment with radiolabelled xenobiotics, enzyme activity measurements in 1997, soil and air temperature measurements in 1997-99. Mika A. Kähkönen performed main part of field work.

Paper V
Mika A. Kähkönen wrote the paper, is the corresponding author and interpreted the results. He planned and performed the experimental work, except for the measurements of pH dependence of hydrolytic enzyme activities in the humus layer of pine forest soil, enzyme activities during the first three of the ten measurement campaigns in 1997-99, precipitation, temperatures of soil and air.

ABBREVIATIONS

AMC	4-methyl-7-amino-coumarin
APHA	American Public Health Association
ATP	Adenosine triphosphate
AOX	Adsorbable organic halogen
AOX-Cl	Adsorbable organic halogen expressed as chlorine
d.m.	Dry matter
d.w.	Dry weight
E	Eastern longitude
EC_{50}	Effective concentration, causing response in 50% of the exposed
EOX	Extractable organic halogen
EOX-Cl	Extractable organic halogen expressed as chlorine
GC	Gas chromatograph
H	Humus layer
IRGA	infra red gas analyser
I-TEQ	Toxicity equivalent
K_{ow}	Octanol-water partition coefficient
ISO	International Organisation for Standardisation
L	Litter layer
MUF	4-methyl-umbelliferyl
MS	Mass spectrometry
N	Northern latitude
TOC	total organic carbon
SIR	Substrate-induced respiration

1. BACKGROUND

1.1. Sources and fate of the halogenated organic matter in soils and sediments

Organic chlorine compounds were formed in pulp and paper industry when kraft pulp is bleached with elemental chlorine or chlorine dioxide. This resulted in discharges of organic bound chlorine into Finnish waters (reviewed in the Ph.D. theses of Jokela 1997, Saski 1998, Suominen 1999 from our laboratory). The discharges have resulted in the accumulation of high amounts of recalcitrant organic chlorine compounds in the sediments of pulp mill recipient waters (reviewed in the Ph. D. theses of Kankaanpää 1997, of Saski, 1998 and of Suominen, 1999, Palm and Lammi 1995, Suntio et al. 1988 a review). Elemental chlorine in pulp bleaching was abandoned in Finland in 1993 and was substituted with chlorine dioxide and nonchlorine chemicals. This together with waste water treatment installed in all pulp and paper mills, decreased the amount of organic chlorine discharges into recipient waters by > 90% in the year 2000 when compared to that in 1980-1989 (Figure 1). The amount of organic bound chlorine discharged into Finnish recipient waters was 930 tons of AOX in the year 2000 (Figure 1) showing that waste waters have become less polluting during the recent past.

Soils around the over 800 former and present Finnish sawmills are contaminated with chlorophenols, polychlorinated furans and dioxins (Laine, 1998). This was caused by the use of the wood preservative Ky5 in most sawmills between 1930 and 1988 as a fungicide and protection against the bluestaining of timber. Ky5 contained polychlorophenols as the active substance and as impurities it contained polychlorinated furans and dioxins (Kitunen, 1990; Valo, 1990). Two-thirds of residents of Finland use tap water distributed by municipal water works. The water consumption in Finnish communities was 240 dm^3 $capita^{-1}$ day^{-1} in the year 2000, including the usage by residients and enterprises (Lapinlampi and Raassina, 2002). The consumption of municipal water thus was $30 * 10^6$ m^3 $year^{-1}$, of which 60% was ground water and 40% surface water. Table 1 displays chlorine containing chemicals potentially usable for disinfection imported and produced in 2001 in Finland. Chlorine is used to disinfect surface water which is made into drinking water. For this purpose mainly sodium hypochlorite and chlorine gas are used in Finland (Table 1). Jokela et al. (1992) reported that the AOX content of Finnish drinking waters was in 1990 > 100 µg dm^{-3} in 70% of the 22 towns using surface water as raw water and < 10 µg dm^{-3} in 90% in waterworks using groundwater as raw water (Jokela et al. 1992). If mean AOX content of chlorinated drinking water was between 20 – 100 µg dm^{-3}, in the range shown by Jokela et al. 1992, this means that the total amount of organic bound chlorine in municipal water may have ranged from 250 to $1250 * 10^3$ kg per year in 2000. This is close to the amount of organic bound chlorine ($930 * 10^3$ kg AOX) discharged into recipient waters from pulp and paper mills in Finland in the year 2000 (Figure 1). The chlorine used for disinfection of the raw water reacts with the organic matter to form organic chlorine compounds, some of which

are mutagenic, for example MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone) (Komulainen et al. 1997). Organic chlorine compounds are used for disinfection of materials other than drinking water, 22 * 10^3 kg in 2001 (Table 1). This is 2 - 9% compared to the total amount organic bound chlorine resulting from chlorine disinfection of municipal tap water. A considerable part (40 %) of tap water, which is treated surface water, can be the source of organically bound chlorine compounds.

Table 1. The imported and produced amount of chlorine containing chemicals potentially usable for disinfection as Cl in 2001 in Finland. Data from Product Register of the Chemical Register (KETU, 2002)

Chemical	CAS-number	Imported in 2001 10^3 Cl kg	Produced in 2001 in Finland 10^3 Cl kg	Total in 2001 10^3 Cl kg
Inorganic chlorine compounds				
Sodium hypochlorite	7681-52-9	346	1333	1679
Calcium hypochlorite	7778-54-3	1.0	3.4	4.4
Chlorine gas	7782-50-5	-	116148	116148
Chlorine dioxide	10049-04-4	-	74497	74497
Total amount of inorganic chlorine compounds		347	191981	192328
Organic chlorine compounds				
Chloroform	67-66-3	6.5	1.8	8.3
Chloramine T	127-65-1	0.6	12.9	13.5
Trichloro isocyanurate	87-90-1	0.14	0.23	0.37
Triclosan (Irgasan DP)	3380-34-5	0.15	0	0.15
Total amount of organic chlorine compounds		7.4	14.9	22.3

Organic chlorine compounds are also produced by natural processes in terrestrial and aquatic ecosystems. Bacteria, lichens, fungi and plants produce halometabolites. Haloperoxidase enzymes catalyse the halogenation of organic compounds in the presence of H_2O_2 and halide ions (Asplund and Grimvall 1992, Baker et al. 2001, Keppler et al. 2002, reviews by van Pee 1996, by Suominen 1999 and Suominen et al. 2001).

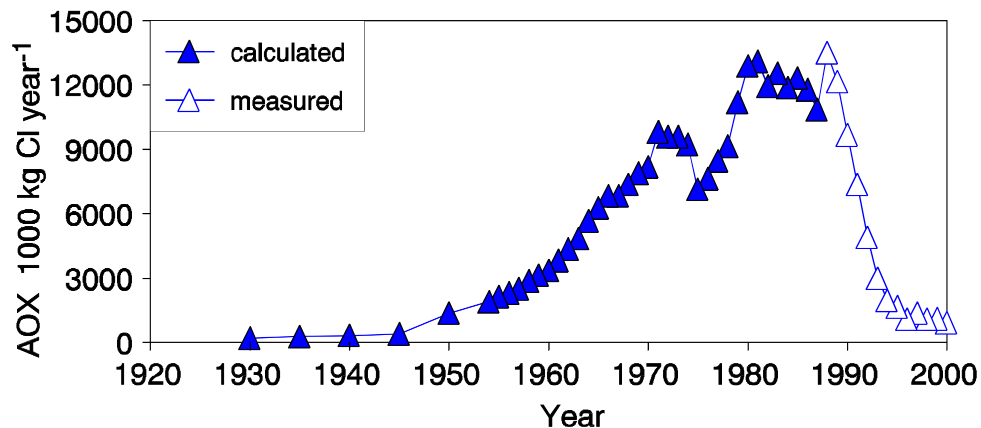


Figure 1. Historical development of AOX discharges from the pulp and paper industry in Finland. The figures for 1989 to 2000 are measured values and those from 1930 to 1988 were estimated based on the assumption that 6% of the chlorine used for bleaching binds to organic matter. The calculations were based on the data of Sieppi (2000),of Harjanne (2001) and of Suominen (1999).

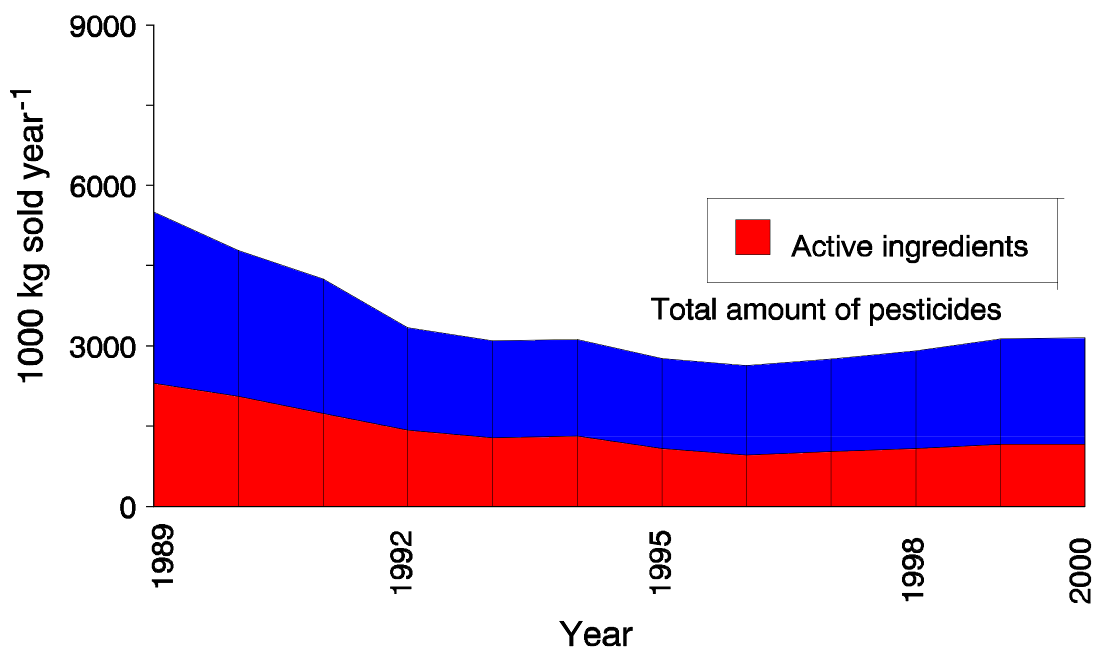


Figure 2. Pesticides and their active ingredients sold in Finland from 1990 to 2000. The figure is based on the data of Hynninen and Blomqvist (1990, 1991,1992, 1993, 1994, 1995, 1996, 1997, 1998, 1999, 2001) and Londesborough et al. (2000).

Organic chlorine compounds are spread by atmospheric fallout (Kanters et al. 1996, Laniewski et al. 1999). Jokela et al. (1992) showed that the 15-month mean of AOX contents in rain water at two sampling sites located about 1 km from the pulp mills in the City of Imatra was 0.55 ± 0.17 and 0.56 ± 0.22 mg Cl m^{-2} $month^{-1}$ in 1988-1989. In the same period the AOX contents in rain water 15 km north-west of the City of Imatra was 0.34 ± 0.15 mg Cl m^{-2} $month^{-1}$ (Jokela et al. 1992) showing that atmospheric fallout can be a source of organically bound chlorine in rural areas.

Many pesticides are organic chlorine compounds. 3100 * 10^3 kg of pesticides were sold in Finland in the year 2000 (Hynninen and Blomqvist 2001). These contained 1160 * 10^3 kg of effective substances (Figure 2). The amount of active ingredients used in pesticides in Finland decreased from 1990 to 2000 by 50% (Figure 2). The pesticides used in year 1990 contained 300 * 10^3 kg of organic bound chlorine and 84 * 10^3 kg in 2000 (Figure 3). These amounts correspond to 3% and 10% of the amount of the adsorbable organic halogen compounds (AOX) discharged from pulp mills into surface waters in Finland in 1990 and 2000, respectively. Pesticides are applied to soils (agricultural, forest) and may leach from there into lakes. The amount of organic chlorine contained in imported chlorine chemicals was about 1500* 10^3 kg in the year 2000 and the same in 1990 (Figure 4). Organic chlorine compounds used as solvents have also contaminated soils (Kolari and Salkinoja-Salonen, 1993). If all imported organic chlorine chemical were eventually released into the environment, the amount of imported organic chlorine chemicals would be equal to 8% of that discharged from the pulp mills in 1990 (9700 * 10^3 kg) and 1.6 fold higher than that discharged by pulp mills in the year 2000 (930 * 10^3 kg). The conclusion of the data in Figures 1-4 and Table 1 is that the relative importance of sources of organic bound chlorine other than from pulp mills has increased during the past ten years, because the pulp bleaching discharges have greatly diminished.

1.2. Biodegradation of organic matter in soils and sediments

1.2.1. Organic matter in the forest soils and lake sediments

Soil organic matter represents the main part of the global organic carbon. Dead organic matter (detritus) in forest soils has been estimated to contain globally 1.5 x 10^{15} kg of carbon, which is 2 – 3 fold more than that in the living biomass in the forests (Zech and Kögel-Knabner 1994). Boreal and temperate forests soils contain large amounts of stored carbon (Adams et al. 1990, Cannell and Milne 1995, Cannell et al. 1996, Liski and Westman 1997a,b, Perruchoud et al. 2000, Prentice and Fung 1990, Prentice et al. 2000). The carbon in forest soils originates from the atmospheric carbon dioxide which is fixed by green plants and other autotrophs. Forest plants release carbon containing photosynthates into the forest soils as above-ground litter, root exudates and below-ground root litter (Figure 5) (Zech and Kögel-Knabner 1994).

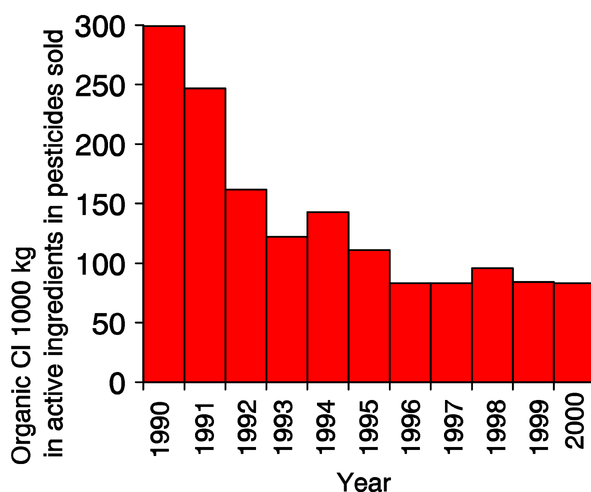


Figure 3. The amounts of organic chlorine compounds in active ingredients sold as pesticides in Finland from 1990 to 2000. The figure is calculated from the data in Fig. 2 using the chemical formulas.

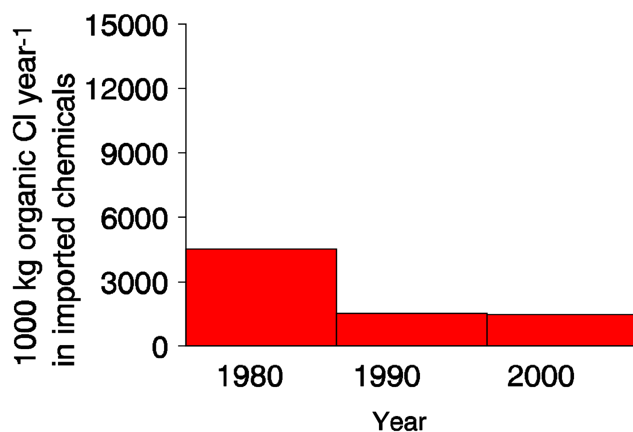


Figure 4. Chlorine imported to Finland as organic chlorine chemicals in the years 1980, 1990 and 2000. This figure summarize the imports of mono-, di-, tri- and tetrachloromethane, 1,2-dichloroethane, 1,2-dichloropropane, 1,1,1,-trichloroethane, vinylchloride, trichloroethene, tetrachloroethene, chlorobenzene, *o*- and *p*-dichlorobenzene. The amounts of imported chlorine were calculated based on molecular weights. Data on the imported amounts of individual chlorine chemicals prior to 1987 are not available, because the statistics system used by the Finnish customs was changed that year. The imported chlorine amounts for 1980 are based on the following assumptions: Saturated chlorofluoro compounds contained carbon, chlorine and fluorine in molar ratios of 1:1:1; dichlorohydrocarbons contained chlorine and carbon in molar ratios of 1:1; the aliphatic chlorohydrocarbons contained chlorine and carbon in a molar ratio of 1:1. This table is based on the data retrieved from the Board of Customs (1980, 1990, 2000).

Organic matter is leached into the forest soils from secondary sources as microbial and animal residues (Figure 5, Zech and Kögel-Knabner 1994). Cellulose, lignin and hemicellulose are the most abundant compounds in forest litter (Brady 1984, Zech and Kögel-Knabner 1994). Humic compounds are nonliving organic matter which are greatly transformed in biochemical and chemical processes from primary and secondary sources (Figure 5, Zech and Kögel-Knabner 1994). Humus compounds are stable and polymerised organic compounds (Atlas and Bartha, 1998). Lignin is a plant biopolymer which consists of phenylpropane subunits linked by carbon-carbon or ether bonds forming a complex three-dimensional structure. Lignin is recalcitrant because its biodegradation is a complex oxidative process (Atlas and Bartha 1998). Carbon returns to the atmosphere as carbon dioxide and methane. Carbon dioxide is the final stage of decomposition and oxidation of organic matter after the detritus has been used as source of energy and nutrients by heterotrophic organisms.

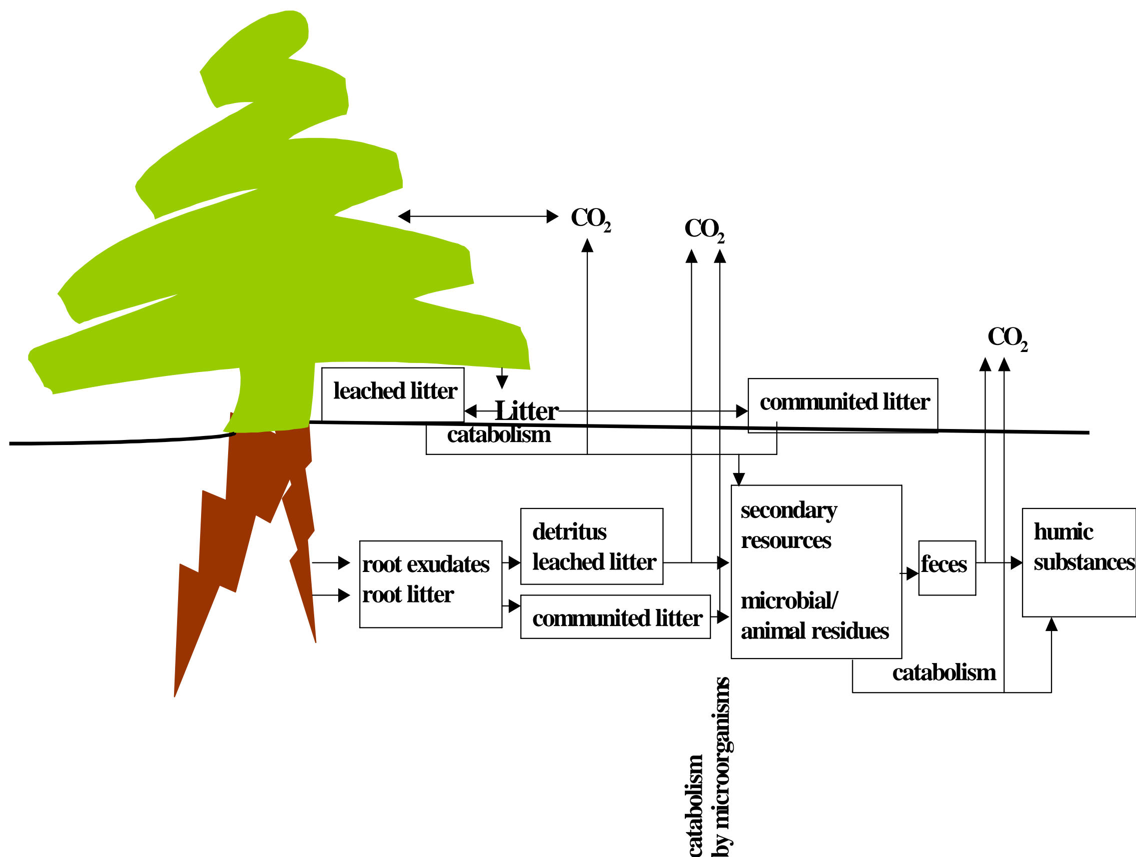


Figure 5. Distribution of organic matter in forest soil. Modified from Zech and Kögel-Knabner, (1994).

Podzol is the soil type typical for boreal coniferous forest. Podzol soil consist of four main layers, the humus layer on the surface followed by the eluvial, illuvial layers and followed by the ground soil towards the depth (Figure 6). The above-ground litter lies on the surface of the humus layer. Water soluble organic compounds in the organic matter form soluble complexes with aluminum and iron enhancing the weathering of solid inorganic materials. Aluminum and iron are sorbed or precipitated deeper in the soil, in the illuvial layer. The precipitation of aluminum or iron is due to an increase of the metal content in the complexes with depth and microbial degradation of the organic ligand or an increased pH of the soil. The red colour of soil in the illuvial layer is caused by the enriched iron oxides. Podzol is formed in soils receiving acid and slowly degrading litter. The result of the latter is that humus accumulates above the mineral soils (reviewed by Lundström et al. 2000a,b and by Schaetzl and Isard 1996).

Lake sediments contain allochtonous and autochtonous organic material from primary and secondary sources, such as humus from the terrestrial runoff. The soil types in the drainage area are important for the quantity and quality of the dissolved organic carbon in the runoff (Kortelainen, 1999). The rates of primary production and organic matter decomposition control the amount of dissolved organic carbon in surface waters (Kortelainen, 1999). Solar radiation photochemically mineralizes or decomposes dissolved organic matter in lakes into substances suitable as substrates for microorganisms (reviewed in the Ph.D. thesis of Vähätalo, 2000). Most of the allochtonous and autochtonous detritus entering lakes will sediment on the lake bottom, where it is slowly mineralised to carbon dioxide, methane and other inorganic compounds (Varnam and Evans 2000, Ehrlich 1990).

Figure 6. Podzol soil profile. The humus layer is on the surface, followed (in depth) by the greyish eluvial layer and the redish illuvial layer (enrichment layer), and the ground soil. The photograph is of Hyytiälä *Pinus sylvestris* soil. Photograph by Hannu Ilvesniemi.

1.2.2. Enzyme activities in the forest soils and sediments

Extracellular hydrolytic enzymes convert the detrital carbon and organic compounds of phosphorus, nitrogen and sulphur into forms available as nutrients to microbes and plants in soils and sediments. The activity of enzymes depends on the temperature, pH, moisture and the concentration of the substrate. Soil and sediment enzymes are of microbial, plant or animal origin (Atlas and Bartha, 1998; Sylvia et al. 1999; Zech and Kögel-Knabner1994).

Hydrolytic enzymes in soils and sediments can form enzyme-organic matter or enzyme-inorganic matter complexes without losing their activity. Enzyme soil complexes have been reported to resist denaturation better than those without soil complexes (Tabatabai and Fu, 1992). Peroxidase and catalase as humic-enzyme complexes in peat soil were more resistant to heat (tested at 50 °C) than the noncomplexed enzymes (Serban and Nissenbaum 1986). Enzymes may be long-preserved: sulphatase activity at the depth of 8 m of peatland, which was dated to be 6000 years old, was 3% of that in the surface (Speir and Ross 1990). Gianfreda and Bollag (1994) showed that phosphatase activity decreased by 20 – 80% when immobilised onto different field soils. After recovery from immobilisation the activities increased to the level close to that before the immobilisation indicating that the immobilized enzymes had been preserved. Rao et al. (1996) showed that phosphomonoesterase formed complexes with clays, organic molecules and organo-mineral complexes. Köster et al. 1997 showed that the activities of α-glucosidase, β-glucosidase, phosphomonoesterase and sulphatase decreased by factors of 2 - 10 from the surface 0- 1 cm sediment to the depth of 9-10 cm in southern Baltic Sea. β-glucosidase activities of marine sediment had a decreasing gradient from the surface towards the depth in Maine in the USA (King 1986). Fabiano and Danovaro (1998) showed that β-glucosidase and aminopeptidase activities of marine sediment close to Antarctica decreased from the surface towards the depth. Wittmann et al. (2000) showed that hydrolytic enzyme activities had a gradient decreasing up to two orders of magnitude from the surface of freshwater sediment towards the depth of 25 – 30 cm. The sediment hydrolytic enzyme activities thus located mainly in the surface of marine and freshwater sediments. Formation of enzyme-organic matter or enzyme-inorganic matter complexes is probably the mechanism which allows enzymes to preserve in sediments and soils during long time as well as in cold seasons.

Soil and sediment enzymes hydrolyse biopolymers to oligomers or monomers. Cellulose is the most abundant polysaccharide in green plants. Cellulose is a linear polymer, consisting of 300 to 15000 glucose units linked by β -1,4-bonds (Figure 7). Cellolytic enzymes hydrolyse cellulose to oligomers or monomers of glucose (reviewed by Tomme et al. 1995 and by Warren 1996). Another pathway to degrade cellulose involves phosphorylated intermediates. Cellulose degradation in soil has been described in detail in the Ph.D. thesis of Kurka (2000).

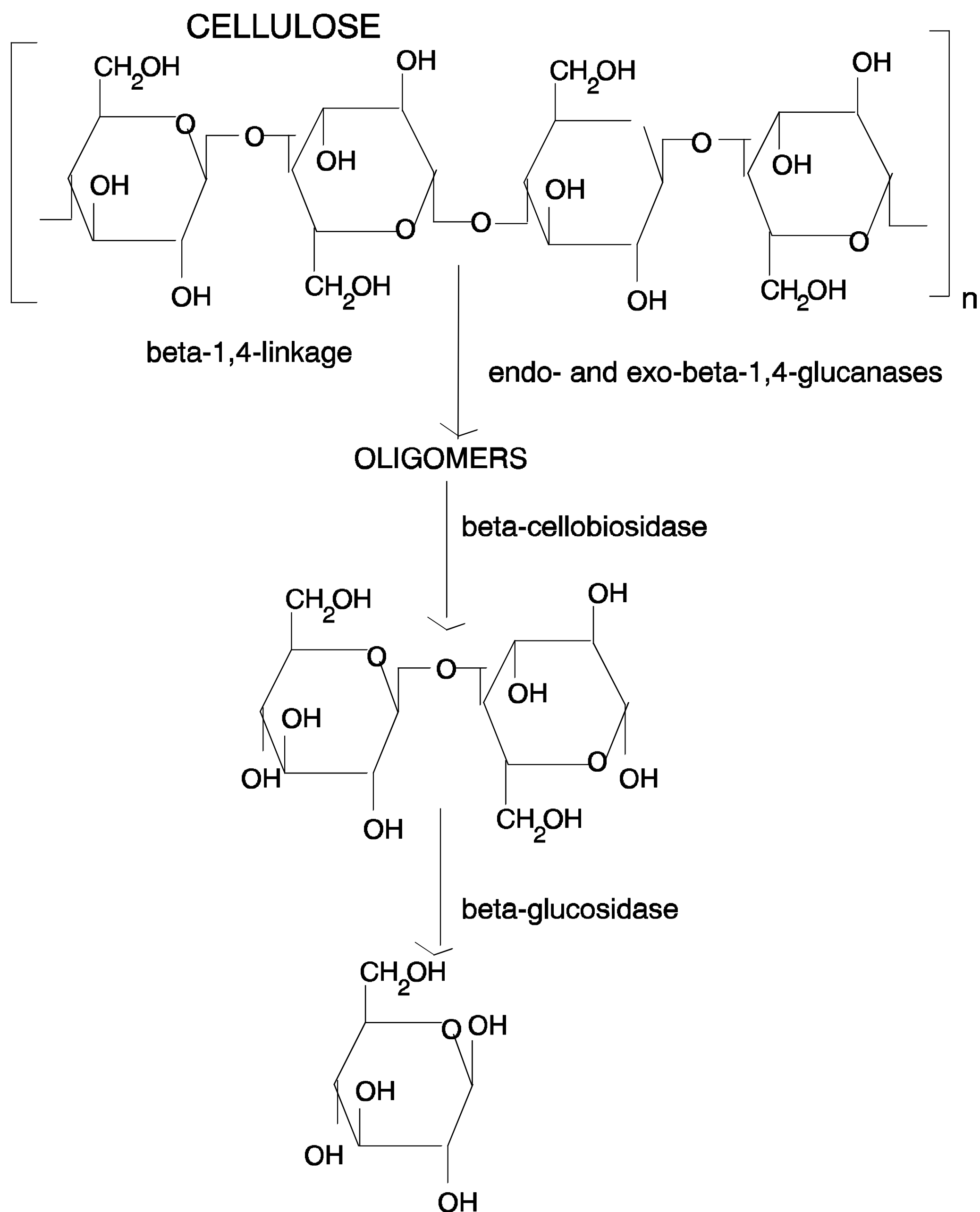


Figure 7. Enzymic hydrolysis of cellulose. Cellolytic enzymes catalyse the hydrolysis of cellulose ultimately to D-glucose. Mineralization of cellulose starts with the loss of its crystalline-like structure then continues with depolymerization. Cellolytic enzymes have generally three different activities, exo-β-1,4-glucanase, endo-β-1,4-glucanase and β-1,4-glucosidase. Exo-β-1,4-glucanases cleave units from the end of the cellulose chain. Endo-β-1,4-glucanases cleave bonds along the cellulose polymer chain. The formed cellobioside and cellotriose are hydrolysed to glucose by β-1,4-glucosidases.

Starch is a storage polysaccharide of plants. The glucose units in starch form linear α-1,4-linked or α-1,6-branched chains. Decomposition of starch to monomers requires many exo- and endo-enzymes (Figure 8) such as α-1,4- and α-1,6-glucosidases (Sylvia et al. 1999, reviews by Tabatabai and Fu 1992 and by Warren 1996). Alternatively, microbes can transform starch by exocellular cyclodextrin glycosyltransferases into cyclodextrins. The formed cyclodextrins are transported into microbial cells and then hydrolysed by cyclodextrinase as reviewed by Pocsi (1999). Starch degradation pathways were described in the recent Ph.D. thesis of Pirttijärvi (2000).

Hemicelluloses are the second most abundant carbohydrate in plants. Hemicellulose molecules consist of 50 – 200 sugar or uronic acid units (Figure 9). Hemicellulose is a branched or linear heteropolymer. Hemicellulose contains various pentoses as xylose and arabinose, hexoses as mannose, glucose and galactose and uronic acids as glucuronic and galacturonic acids. Due to the heterogenity of the sugar units, several different exo- and endo-enzymes are involved in the hydrolysis of hemicellulose molecules to oligomers and monomers (Sylvia et al. 1999, review by Warren 1996).

Chitin is a polymer of N-acetyl-glucosamine linked by β-1,4-bonds (Figure 10). Chitin is hydrolysed to oligomers and further to monomers (Atlas and Bartha 1998, review by Tabatabai and Fu 1992). Chitin is the major polymer of the exoskeleton of insects, crustaceans and the cell walls of fungi.

Proteins are hydrolysed by proteinases into peptides. The peptides formed are hydrolysed by peptidases into single amino acids (Sylvia et al. 1999, review by Tabatabai and Fu 1992). Esters, such as waxes, are hydrolysed by esterases that cleave the ester linkages between the organic acid and the alcohol (reviewed by Manafi et al 1991).

Phosphatases and sulphatases hydrolyse esters of phosphoric and sulphuric acids, respectively. Phosphatases are a group of enzymes expressing phosphomonoesterase, phosphodiesterase or phosphotriesterase activities (Alef and Nannipieri 1998). These catalyse the hydrolysis of phosphate from mono-, di- and trisubstituted phosphate esters, respectively. Sulphatases hydrolyse aliphatic and aromatic organic sulphate esters. Sulphatase releases inorganic sulphate by splitting the O-S-bond (Alef and Nannipieri 1998, review by Tabatabai and Fu 1992).

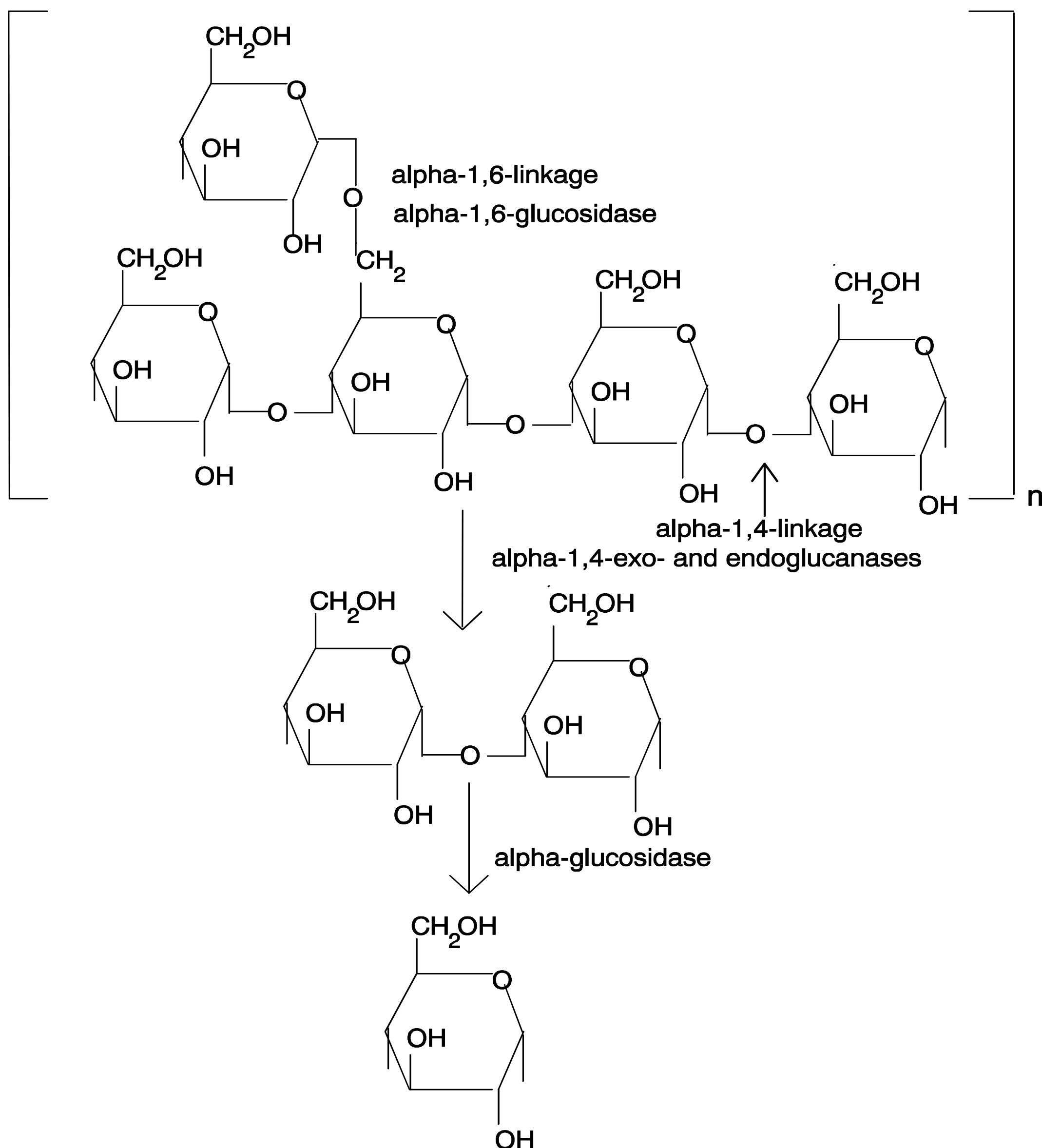


Figure 8. Hydrolysis of the α-1,4- and α-1,6-bonds in starch. Amylose is a linear polymer of glucose units linked by α-1,4-bonds. Amylopectin is a branched polymer in which glucose units are linked by α-1,4- and by α-1,6-bonds. α-1,4-exo- and α-1,6-glucanases (amylases) hydrolyse amylose and amylopectin to disaccharides. α-1,4-glucosidase hydrolyses disaccharide (two-glucose units) to glucose.

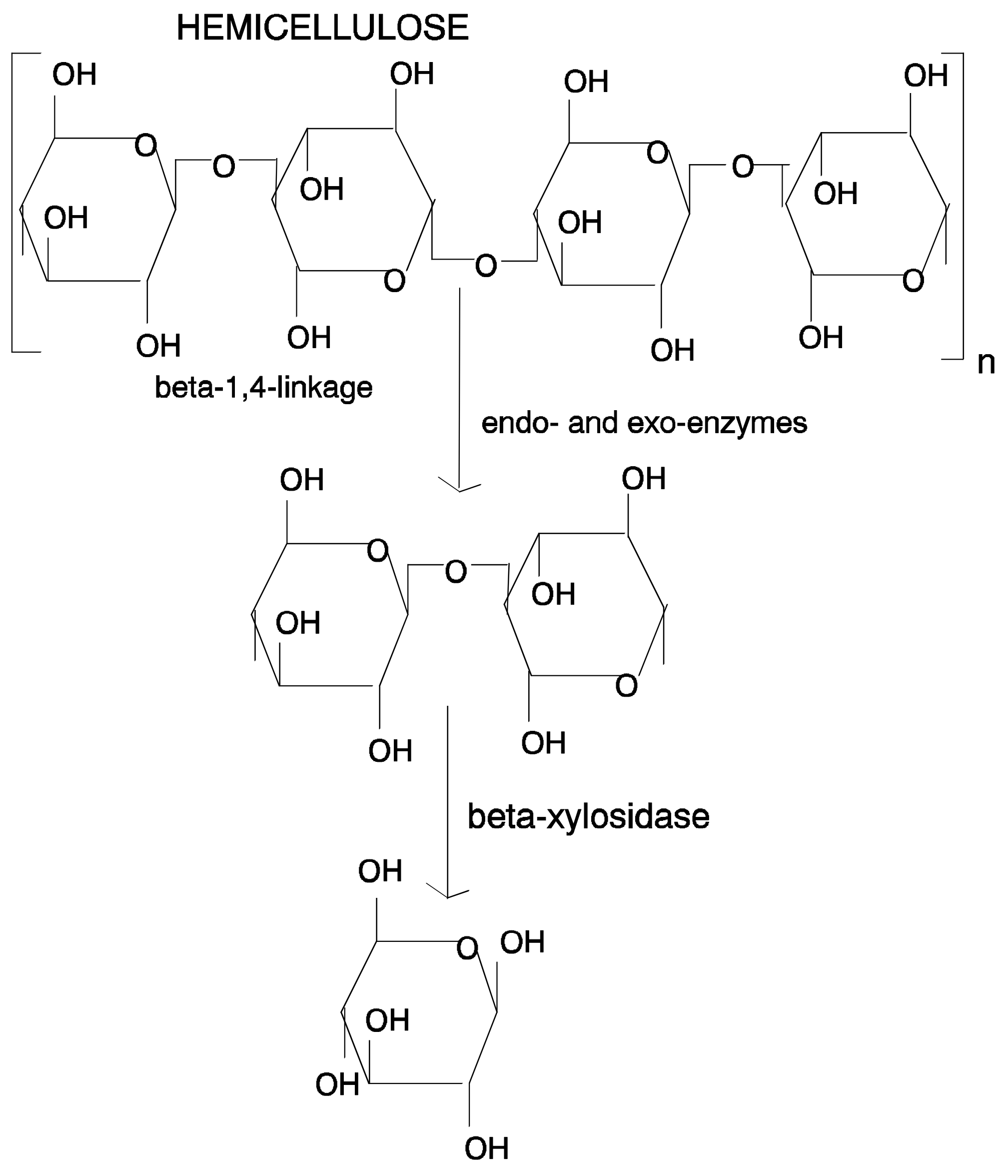


Figure 9. Hydrolysis of hemicellulose by exo- and endo-xylosidases cleaving β-1,4-bonds between xylose units. Hemicellulose molecule can be branched or linear. Many exo- and endo-enzymes are involved in the depolymerization of hemicellulose molecules due to the heterogenity of its sugar units.

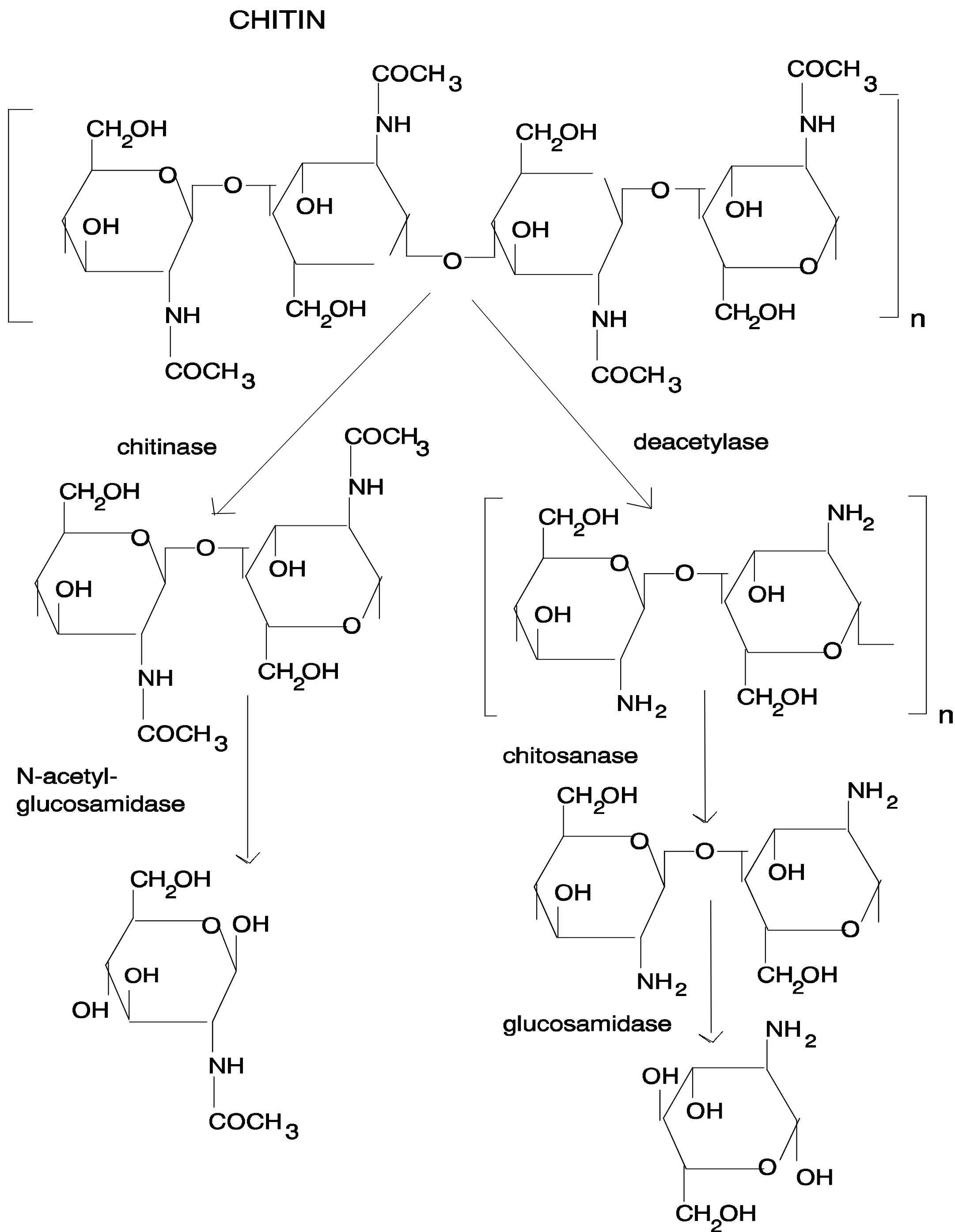


Figure 10. Degradation of chitin by hydrolysis of β-1,4-bonds. Chitin is hydrolysed by exo- and endo-chitinases finally to dimers (diacetylchitobiose). N-acetylglucosamidase hydrolyses dimers to N-acetyl-glucosamine units (left). An alternate route is that chitin is deacetylated to chitosan (right) before depolymerization. Exo- and endo-chitosanases hydrolyse chitosan to dimers (chitobiose). Glucosaminidase hydrolyses the dimers to glucosamine monomers.

1.2.3. Fluorogenic model substrates for assessing hydrolytic enzyme activities in environmental samples

Figure 11 displays the three main stages in the luminescence process. These are the excitation, the excited state and the fluorescence or phosphorescence emission (Campbell 1988). Energy for the excitation is provided by an external source. As the fluorophore absorbs energy, it transforms into an excited electronal singlet state. The excited-state fluorophore can change conformation or interact with other molecules in its surroundings. These processes cause decrease of energy with non-radiative transitions in the excited state producing a relaxed singlet or triplet states, where emission of light quanta starts. Electrons transform between singlet states in fluorescence. Electrons transform between the singlet and triplet state in the excited-state in phosphorescence. The transition between singlet and triplet state decreases the rate of the emission process, thus transition between triplet and singlet states is hundred to million fold slower than the transition between two singlet states. Light is emitted when the fluorophore returns to the ground state. The emitted light has a longer wavelength and lower energy content than the excitation light, thus the energy of the excited-state is partly dissipated. The emitted photons can be measured separately from the excited photons due to their wavelength differences, this increases the sensitivity of the fluorescence technique.

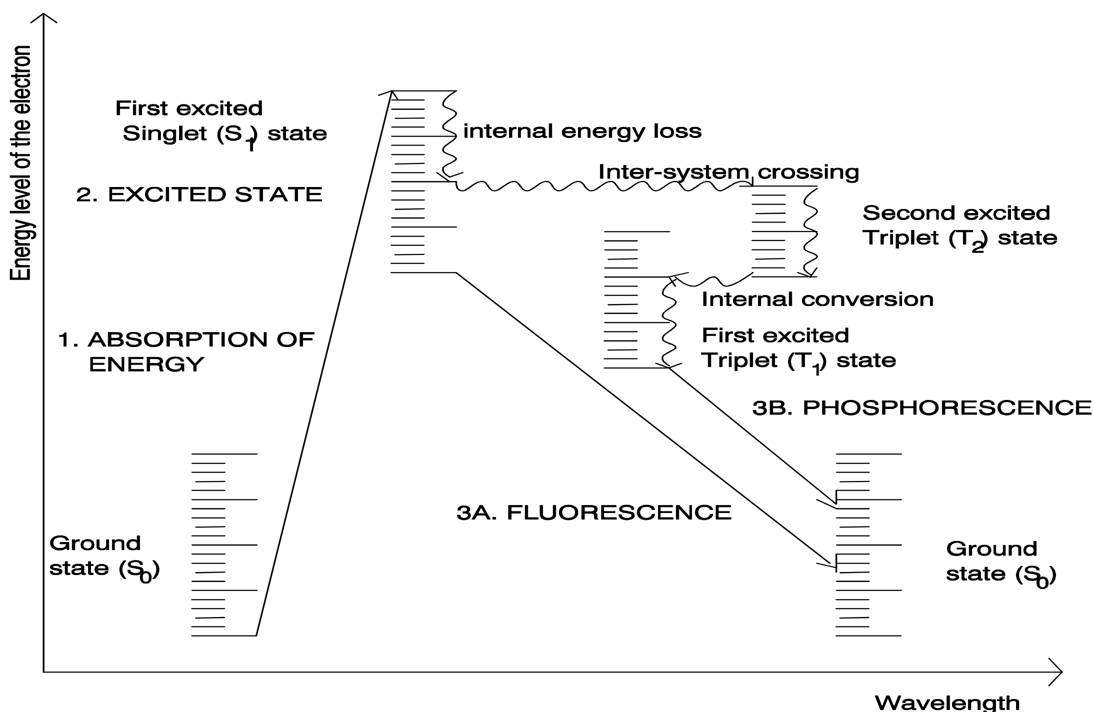


Figure 11. Energy transitions of electrons in the luminescence processes. Absorption of energy (1), the excited state (2) and the emission of fluorescence (3A) and the phosphorescence (3B) (Modified from Campell, 1988).

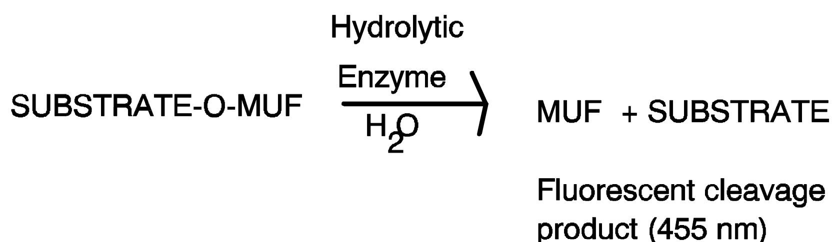


Figure 12. Hydrolytic enzymes cleave 4-methylumbelliferyl (MUF) conjugated substrates to the fluorescent product, methylumbelliferone (MUF).

Commercially available fluorogenic surrogate substrates used to assay hydrolytic enzymes are for example 4-methylumbelliferyl (MUF) and 4-methyl-7-amino-coumarin conjugated (AMC) compounds (Figure 12). MUF and AMC are excited using light of 360 nm and the emission measured at 455 nm. Kinetic fluorometry with fluorogenic model substrates allow for rapid (3 to 60 min) and sensitive analysis of environmental samples for hydrolytic enzyme activities. The activities can be measured from single samples with a fluorometer or from multiple samples using microtiter plates with a kinetic fluorometer (Table 2). It is possible to measure the fluorescent products in the presense of unreacted substrates (review by Manafi et al. 1991). The assay using chromogenic substrates or product derivatives (Table 2) is less sensitive by a factor 25 – 100 (review by Tabatabai and Fu 1992) and therefore requires longer incubation times (up to 24 hours) than when fluorogenic model substrates are used.

Table 2. Measurement of hydrolytic enzymes in environmental samples.

Methods	Product detected	References
Fluorogenic methods	fluorescent product	Chrost 1991
Chromogenic methods	colored product	Alef and Nannipieri 1998
		Tabatabai and Fu 1992
Product derivative methods	product derivative	Tabatabai and Fu 1992
		Tabatabai et al. 2002

1.2.4. Sources and sinks of methane in sediments and forest soils

Methane and carbon dioxide are formed as end products of organic matter mineralization in soils and sediments. Many types of lands are sources of methane (Figure 13): rice fields, wetlands, peatlands and landfills (reviewed by Conrad 1995, 1996, by Frenzel 2000, by Frenzel and Karofeld 2000). Well aerated soils may turn anaerobic after water logging during snow melt or heavy precipitation. The soil may then change from a methane sink to a methane source (reviewed by Frenzel and Karofeld 2000, by Segens 1998, Smith et al. 2000). Rice fields and wetlands are rich in organic detritus. Aerobic mineralization of detritus depletes the soil of oxygen. The soil becomes anoxic and its redox potential becomes negative. This creates an environment suitable for methane producing archae (Chidthaisong and Watanabe 1997, reviewed by Frenzel 2000 and by Segens 1998). Methane sources may also be anthropogenic, such as landfill sites and leakages from gas pipelines, the drilling for natural gas and the burning of coal.

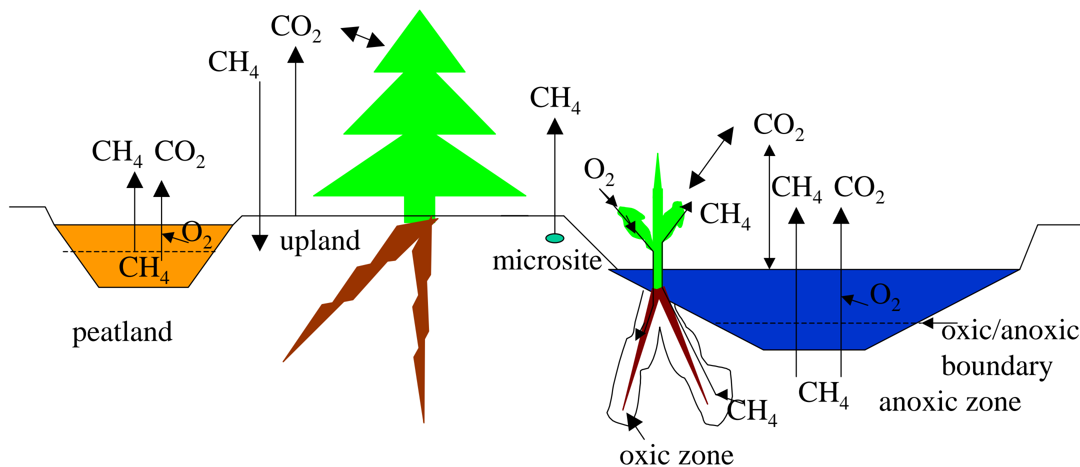


Figure 13. Sources and sinks of methane and carbon dioxide in soils and sediments.

Methane is transported from soils and sediments into the atmosphere by diffusion, bubbling and plant mediated transport. The atmospheric content of methane is presently 2 ppm. The aerobic surface in peatlands, sediments of lakes and rivers can contain methanotrophic microbes converting these zones into methane sinks, thus preventing methane entering the atmosphere (reviews by Conrad 1995, 1996, by Segens 1998, by Frenzel 2000, by Koschorreck 2000). It is estimated that up to 80% of the methane formed in rice fields becomes oxidized by microbial activity before methane reaches the atmosphere (reviewed by Frenzel 2000).

Plants can increase or decrease methane oxidation and emission from soils and sediments. Methane may be channelled through plants into the atmosphere, bypassing the aerobic methane oxidizing zone (reviewed by Conrad 1995, 1996 and by Frenzel 2000). Plants can increase methane production providing substrates in the form of root exudates to the methanogens. Plants can also transport oxygen from the atmosphere and release it into the root zone (Segens 1998) thus providing oxygen to the submerged soils. Methanotrophs oxidize methane in the aerobic root zone (reviews by Segens 1998 and by Frenzel 2000).

Methane is oxidized in many different types of well-aerated soils, from the tropics to the tundra (Figure 13) (Amaral et al. 1998, reviewed by Conrad 1996, by Hansson and Hansson 1996 and by Mancinelli 1996, Saari et al. 1998). Oxidation of atmospheric methane in soil is mainly limited by diffusion. Diffusion of methane is faster in dry than in wet soil (Bowden et al. 1998, Striegl 1993, Keller and Reiners 1994).

Methanotrophic bacteria are α-proteobacteria. A few species of yeasts also oxidize methane. Bacteria oxidize methane to carbon dioxide over methanol and formaldehyde intermediates (reviewed by Bedard and Knowles, 1989). The enzyme oxidizing methane to methanol is methane mono-oxygenase (MMO), which can be in particulate or in soluble form. The soluble form of methane mono-oxygenase is synthesized in an environment where sufficient copper is present (reviewed by Bedard and Knowles 1989, Dalton 1992, by Lipscomb 1994). Formaldehyde is assimilated by the microbe or it is oxidized by formaldehyde dehydrogenase to formate and further to carbon dioxide (reviewed by Bedard and Knowles, 1989).

1.2.5. Influence of land use on methane oxidation

Methane oxidation activity of soil is affected by land use. Willison et al. (1995) reported that methane oxidation activity was higher in woodland ($2.0 \cdot 10^{-3}$ µmol of CH_4 dm^{-3} h^{-1}) than in grassland ($0.9 \cdot 10^{-3}$ µmol of CH_4 dm^{-3} h^{-1}) and lowest in arable soils ($0.4 \cdot 10^{-3}$ µmol of CH_4 dm^{-3} h^{-1}) in the United Kingdom. These three differently used lands were sinks for methane. Methane oxidation activity was 13 µmol of CH_4 m^{-2} h^{-1} in the surface layer of an intact soil core (12 cm) has been reported for German forests (Hutch 1998). This is one order of magnitude higher than in German field soils (0.5 – 0.6 µmol of CH_4 m^{-2} h^{-1}) and two orders of magnitude higher than in ploughed field soil (0.10-0.14 µmol of CH_4 m^{-2} h^{-1}), when measured with 10 ppm CH_4 at 25 °C. These data of Hutsch (1998) and those of Willison et al. (1995) indicate that the economic use of land may depress the methane oxidation activity in the soil.

Table 3 summarizes the results from studies on the effects of nitrogen fertilization and atmospheric fallout of nitrogen on methane oxidation in grassland, arable soils, forest soils, wheat and rye fields. Long term fertilization with ammonium salts inhibited methane oxidation more than did fertilization with nitrate or farmyard manure (Table 3). Saari et al. (1997) showed that

the mean methane oxidation activity ($0.089 \cdot 10^{-3}$ µmol of CH_4 g dwt soil^{-1} h^{-1}) was three fold higher in mineral soil in nine Finnish *Pinus sylvestris* stands (0 – 5 cm, *in situ* pH = 4.3 ± 0.1) than in mineral soil in thirteen Dutch coniferous forests ($0.028 \cdot 10^{-3}$ µmol of CH_4 g dwt soil^{-1} h^{-1}, 0 – 5 cm, *in situ* pH = 3.5 – 3.8). The atmospheric fallout of nitrogen on these Dutch sites was higher (5 – 9.2 g N m^{-2} year^{-1}) than on the Finnish sites (0.3 – 3.6 g N m^{-2} year^{-1}) indicating that the small natural methane oxidation activity on temperate Dutch coniferous forest soils can be suppressed by high atmospheric fallouts of nitrogen (Saari et al. 1997). Börjesson and Nohrstedt (1998) showed that nitrogen fertilization (0 – 600 kg N as urea ha^{-1}) more than 20 years earlier had no impact on methane oxidation in Swedish *Pinus sylvestris* forests at two sites. One of these two sites had a higher methane oxidation activity at the depths of 7.5 – 15 cm in the mineral soil of a forest fertilized with urea (600 kg ha^{-1}) 27 years previously ($0.430 \cdot 10^{-3}$ µmol of CH_4 g dwt soil^{-1} h^{-1}) than in a non-fertilized forest in the same area. Börjesson and Nohrstedt (1998) showed that in this case methane oxidation correlated positively with the total carbon content of soil. This indicates that nitrogen fertilization increased the methane oxidation potential of the nitrogen limited soils or that the fertilizer-contained nitrogen was rapidly taken up from the forest soil by the trees and the ground vegetation. Therefore the amount of nitrogen remaining in the soil may have been too low to inhibit methane oxidation. The results by Börjesson and Nohrstedt (1998) indicate that single N-fertilization did not neccessarily inhibit methane oxidation in boreal coniferous forest soils. The inhibition of methane oxidation by ammonia observed in the soils is believed to be due to competition between ammonia and methane as substrates for methane monooxygenase. Methanotrophs can oxidize ammonia at a rate of 1/20 of the lowest rate measured for ammonia oxidizers (reviewed by Bedard and Knowles 1989) producing nitrite from ammonia. Nitrite is toxic to methane oxidizing bacteria and will thus decrease the rate of methane oxidation by methanotrophs (Jollie and Lipscomb 1991, King and Schnell 1994). Whalen (2000) showed that sodium nitrite inhibited methane oxidation more severely than did ammonium chloride or sodium nitrate in upland boreal forest soil and in tundra soil. Methane oxidation in the soils did not recover within 30 d after sodium nitrite, sodium nitrate or ammonium chloride was added (Whalen 2000). The conclusion is that long term nitrogen fertilization reduces the consumption of atmospheric methane in the soils and consequently the methane content in the atmosphere, but a single nitrogen fertilization does not necessarily adversely affect methane oxidation in soil.

Table 3. The influence of nitrogen fertilization and atmospheric fallout of nitrogen on methane oxidation activity in different type of soils.

Type	Fertilization or atmospheric fallout	CH_4 oxidation h^{-1}	Reference
Grassland in UK	138 years with 96 kg NH_4^+-N ha^{-1} $year^{-1}$	< $0.0003 \cdot 10^{-3}$ µmol of CH_4 dm^{-3}	Willison et al. 1995
Grassland in UK	138 years with 96 kg NO_3^--N ha^{-1} $year^{-1}$	$1.6 \cdot 10^{-3}$ µmol of CH_4 dm^{-3}	Willison et al. 1995
Grassland in UK	No	$1.6 \cdot 10^{-3}$ µmol of CH_4 dm^{-3}	Willison et al. 1995
Arable soils in UK	140 years with 144 kg NH_4^+-N ha^{-1} $year^{-1}$	$0.06 \cdot 10^{-3}$ µmol of CH_4 dm^{-3}	Hutch et al. 1993
Arable soils in UK	No	$0.6 \cdot 10^{-3}$ µmol of CH_4 dm^{-3}	Hutch et al. 1993
Rye field in Germany	40 years with 75 kg K, 24 kg P, 60 kg NH_4^+-N ha^{-1} $year^{-1}$	2.3 µmol of CH_4 m^{-2}	Hutch 1996
Rye field in Germany	40 years with 75 kg K, 24 kg P ha^{-1} $year^{-1}$	8 µmol of CH_4 m^{-2}	Hutch 1996
Rye field in Germany	farmyard manure (60 kg N, 20 kg P, 60 kg K ha^{-1})	3.4 µmol of CH_4 m^{-2}	Hutch 1996
Maize field in Germany	40 years with 75 kg K, 24 kg P, 60 kg NH_4^+-N ha^{-1} $year^{-1}$	0.38 µmol of CH_4 m^{-2}	Hutch 1996
Maize field in Germany	40 years with 75 kg K, 24 kg P ha^{-1} $year^{-1}$	3.8 µmol of CH_4 m^{-2}	Hutch 1996
Maize field in Germany	farmyard manure (60 kg N, 20 kg P, 60 kg K ha^{-1})	1.3 µmol of CH_4 m^{-2}	Hutch 1996
Thirteen coniferous forest soil in the Netherlands	Atmospheric fallout: (5 – 9.2 g N m^{-2} $year^{-1}$)	Mean $0.028 \cdot 10^{-3}$ µmol of CH_4 g dwt $soil^{-1}$	Saari et al. 1997
Nine *Pinus sylvestris* forest soil in Finland	Atmospheric fallout: (0.3 – 3.6 g N m^{-2} $year^{-1}$)	Mean $0.089 \cdot 10^{-3}$ µmol of CH_4 g dwt $soil^{-1}$	Saari et al. 1997

1.2.6. Measuring carbon dioxide production, formation of methane and methane oxidation in soils and sediments

Microbes in soils and sediments mineralize organic compounds to carbon dioxide, methane and inorganic compounds. A part of the organic carbon is assimilated to microbial biomass. Microbial biomass carbon as a percentage of soil total carbon in birch, spruce and pine soils in Kivalo in Northern Finland was 2.5 %, 2.0 % and 1.7 %, respectively (Smolander and Kitunen 2002). Aerobic mineralization activity can be measured as the production of carbon dioxide or the consumption of oxygen (Table 4). Measuring carbon dioxide production is more sensitive than measuring oxygen consumption, because the atmospheric content of oxygen is almost one thousand fold higher (21%) than that of carbon dioxide (0.036%). The content of carbon dioxide or methane can be analysed from head space of incubation flasks or chambers. A static chamber or a closed bottle (Table 4) is used in short term measurements. Flow-through chambers (Table 4) are needed for long term measurements. The carbon dioxide evolved originates from both microbial and root respiration in chamber measurements. Methane oxidation and carbon dioxide evolution in individual layers of soils can be measured by incubating slices of soil cores in closed bottles. Carbon dioxide emitted from sliced soil layers into the head-space of closed bottles includes the carbon dioxide from the mineralization of soil carbon compounds and may also include carbon dioxide from the mineralization of fresh root litter and root respiration, thus the broken roots may continue to respire for a while. This is a natural component of carbon dioxide emission from the soil. Carbon dioxide evolution from sieved soils, expressed on basis of soil organic carbon, can be used to measure the mineralization rate of carbon in soil. Chamber methods allow the measuring of net emission of carbon dioxide and methane from soil, i.e. the sum of formation and consumption. Chamber measurements have been used to measure respiration in forest soil (Wang et al., 2001), in peat land (Sund et al., 2000a) and in agricultural soil (McGinn and Akinremi, 2001). The eddy covariance method is suitable to measure net exchange of carbon dioxide in a whole ecosystem (Table 4). Eddy covariance measurements are performed above the forest canopy (Markkanen et al. 2001).

Table 4. Experimental set-up for measuring mineralization, soil respiration, net exchange of carbon dioxide, oxidation of and formation of methane.

Test setup	Measured parameters	References
Eddy covariance	CO_2	Markkanen et al. 2001
Chamber methods		
- static	CO_2, CH_4	Gao and Yates 1998
- flow-through	CO_2, CH_4	McGinn and Akinremi 2001
		Pumpanen et al. 2001
Closed bottles	CO_2, $^{14}CO_2$, CH_4	Alef and Nannipieri 1998
Microtiter plate	$^{14}CO_2$	Fulthorpe et al. 1996

Methods commonly used for measuring carbon dioxide, methane and oxygen are listed in Table 5. Carbon dioxide can be measured with a gas chromatograph equipped with a thermal conductivity (TC) detector or with an infra red gas analyser (Table 5). Carbon dioxide can also be measured by absorbing it in an aqueous solution of NaOH or other bases and then titrating with acid. Methane content in the headspace of closed bottles or chambers can be measured with a gas chromatograph equipped with a flame ionisation detector or with an infra red detector.

^{14}C-radiolabelled substrates can be used to measure mineralization of specific compounds allowing the use of microtiter plates (Fulthorpe et al. 1996). The substrate can be a compound uniformly ^{14}C labelled or labelled at specific positions of the carbon skeleton. Mineralization of many types of radiolabelled substrates, for example amino acids, glucose or xenobiotics such as herbicides, pesticides and chlorinated organic compounds, can be studied in this way. The produced $^{14}CO_2$ can be trapped as $Ba^{14}CO_3$ on a filter paper presaturated with $Ba(OH)_2$ (Fulthorpe et al. 1996). The precipitate can be measured with radiographic techniques. One such technique is an erasable imaging plate (IP) (Ameniya and Miyahara 1988, Kohda and Miyahara 1992 and Bara et al. 1993). The radiation from the paper ionizes Eu^{2+} to Eu^{3+} emitting a luminescence, whose intensity is proportional to the absorbed energy of radiation. The emitted luminescence can be transformed to a digital image and processed using computer software. A radiolabelled substrate allows measuring the mineralization of small amounts of substrates, down to the nanomolar level.

Table 5. Methods for measuring carbon dioxide, methane or oxygen contents in closed bottles, static or flow-through chambers.

Test systems	Measurements	References
CO_2 trapped with NaOH	titrated with HCl	Alef and Nannipieri 1998
Infrared detection of CO_2 (IRGA)	CO_2	Alef and Nannipieri 1998
Gas chromatographic detection	CO_2	APHA 1998
^{14}C labeled organic compounds	$^{14}CO_2$	Fulthorpe et al. 1996
Gas chromatographic detection	CH_4	APHA 1998
Gas chromatographic detection	O_2	APHA 1998

1.2.7. Temperature dependence of biodegradation in soil

The law of Arrhenius describes the temperature dependence of the rate of (bio)chemical reactions as shown in the equation (1)

$$k = A e^{-E_a/(R T)} \qquad (1)$$

k = activity when temperature = T (K), R = gas constant (= 8.314 J mol^{-1} K^{-1}), E_a = the energy of activation (J mol^{-1}) and A = Arrhenius constant. If log k is plotted as a function of 1/ T, the energy of activation (E_a) can be calculated from the slope (E_a/ R). E_a and A are constants, when the temperature range is not too wide (Schwarzenbach et al. 2003).

Q_{10}-value is the quotient of activity increase that corresponds to a temperature increase of 10 °C. Q_{10}-values are often used to express temperature dependence of activities.

The law of Arrhenius reflects the activities of biological systems only in a narrow temperature range. Microbial growth and enzyme activities decrease due to inactivation of the enzyme proteins, when the temperature exceeds the maximum tolerated temperature. Structures of proteins and lipids structures change close to the minimum temperatures allowing growth and enzyme activity. Organisms adapt to low temperature by two mechanisms (Margesin and Schinner 1999, reviewed by Puhakka and Melin 1998 and by Robinson 2001). When the temperature decreases, organisms preserve the fluidity of their membranes by increasing the proportion of unsaturated fatty acids, or shortening their chain length or by replacing iso fatty acids by the corresponding anteiso in the membrane lipids. Enzymes produced at low temperature have lower temperature optimum than those produced at higher temperature (Varnan and Evans, 2000). Water solubility of substrates usually increases with temperature. Therefore the availability of the substrates depends on the temperature. Evaporation of water from the soil increases with increasing temperature, affecting soil moisture. Water balance in soils also depends on precipitation, content of organic matter and soil structure (Brady 1984). Solantie (2000) divided Finland into the western thin and the eastern thick frost areas and the southern slush and northern snow zones (Figure 14). Snow cover of soil keeps soil warmer and decreases the penetration of frost. Frost in soil impairs the availability of water and therefore decreases the survival and activities of microbes. Cold inactivation of enzymes is explained by structural changes and below the freezing point by loss of liquid water. McDowell et al. (2000) showed with an *in situ* chamber that 17% of the annual carbon dioxide evolution (65 mol m^{-2} $year^{-1}$) occurred during the snow covered period in coniferous forest soil in Idaho USA. Prieme and Christensen (1997) showed that methane was oxidized in soil cores incubated in the laboratory at – 2 °C. Neal (1990) reported phoshomonoesterase activities at –5 °C, which were 23 – 24% in the humus layer and 52% in the below situated mineral soil of the activities measured at 5 °C. Also McDowell et al. (2000), Prieme and Christensen (1997) and Neal (1990) reported carbon dioxide evolution, methane oxidation and hydrolytic enzyme activities in cold soil.

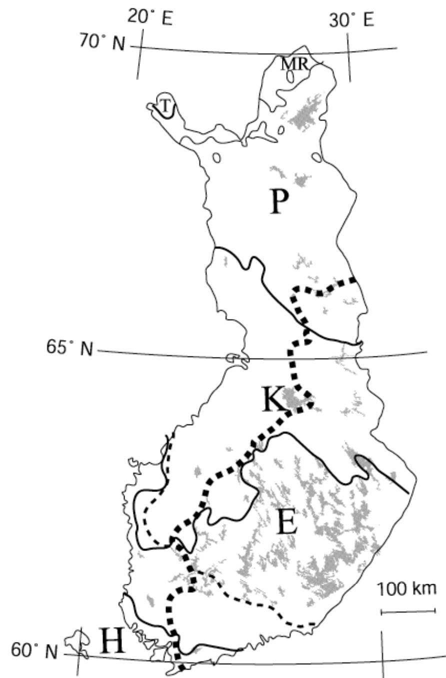


Figure 14. The thickness of frosts in Finnish soils. The thick broken borderline divides Finland into an eastern thin frost area and a western thick frost area (Solantie 2000). The climate zones are marked as follows. H = hemiboreal zone, E = Southern boreal zone, K = Middle boreal zone, P = Northern boreal zone, MR = Timber line, T = Tundra. The narrow broken border line divides Finland to the southern slush and northern snow areas. Graph by Reijo Solantie.

1.2.8. Methane and carbon dioxide are important greenhouse gases

The concentrations of carbon dioxide and methane in the atmosphere have increased during the past decades (reviewed by Conrad 1995, by Frenzel, 2000 and by Körner 2000). Many models predict that this will increase the temperature of the earth's atmosphere. An increased temperature may influence the global carbon cycling (Chiais et al. 2000, Conrad 1995 a review, Foley et al. 2000, Körner 2000, Prentice 2000). Kirschbaum (1995, 2000) predicted that particularly in the boreal region an increase of 1 °C in the temperature can add 35 – 70% to the evolution of carbon dioxide from soil. An increasing soil temperature may lead to an increase of the carbon dioxide content in the atmosphere and subsequently to global warming.

The atmospheric methane concentration increased during past decades by up to 1% annually (Blake and Rowland 1988, Dlugokency et al. 1994). The main sink for the atmospheric methane is chemical oxidation by the OH^- radical (reviewed by Conrad 1995; 1996 and by Frenzel 2000). The concentration of methane in the atmosphere is less than 1% of that of carbon dioxide. The

warming capacity of methane is 26 fold higher than that of carbon dioxide (mol methane / mol carbon dioxide). The capacity of carbon dioxide, methane and dinitrogen oxide, greenhouse gases, respectively to absorb energy from sun light is higher than that of many other atmospheric gases (reviewed by Conrad, 1995; Lelieveld et al., 1993). About 30% of the quantum emission of sun light is reflected from the atmosphere to outer space. The remaining part of the energy is absorbed by the atmosphere and the earth (Schneider 1989). Effectively preventing the increase of atmospheric methane content may appear more effective in preventing atmospheric warming than what can be achieved by slowing the increase in the carbon dioxide content.

1.2.9. Soil pH

Soil pH is usually measured from soil suspension in water. pH(H_2O) shows the content of protons in soil water. The addition of a neutral salt (e.g. $CaCl_2$) before pH measurement will show the bound protons in soil particles (Alef and Nannipieri, 1998). Soil particles are usually negatively charged, neutralized by positive ions such as H^+ or metal ions (Atlas and Bartha 1998). The pH-value after addition of salt reveals the bound acidity. The difference between pH (H_2O) and pH ($CaCl_2$) may vary between the layers of stratified soils. Acid detritus makes forest soils acid. The pH (H_2O) of Finnish forest soils at 65 sites in southern Finland ranged from 3.6 – 5.5 in humus layer and 3.6 – 5.4 in mineral soil at the depth of 0 – 5 cm (Tamminen and Starr, 1990).

Low and high pH in soil cause stress selection of micro-organisms. Procaryotic and eucaryotic organisms maintain a constant pH close to neutral inside the cell. Acidophilic microorganisms prefer acid and alkaliphilic micoroorganisms alkaline environments, but in spite of the extreme pH values of their environments, the intracellular pH is close to neutral. Some microorganisms acidify their environment by producing acids.

1.3. Bioluminescence based analysis of ecotoxicity in soils and sediments

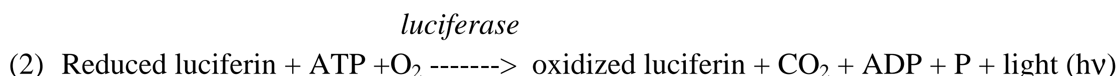
Tests, based on the naturally bioluminescent marine bacterium, *Vibrio fischeri*, have been developed (Table 6) to assess ecotoxicity in sediments (Ringwood et al. 1997), freshwaters, ground waters and soils (ISO, 1998). These tests were recently reviewed in the Ph. D. Thesis of Ahtiainen (2002) The luminescent emission of the marine bacterium *V. fischeri* is related to the energy state of the cell. The emission of light by *V. fischeri* is one of the end points of metabolic pathways in which ATP is consumed (James et al. 1999, England 2001, reviewed by Meighen and Dunlap 1993). Ecotoxicity tests are based on the inhibition of light emission, indicating damaged energy metabolism in *Vibrio fischeri*. Another bioluminescence based toxicity assay is based on a genetically modified strain of *Pseudomonas fluorescens*, a terrestrial bacterium into which *lux* genes have been inserted. The *Pseudomonas fluorescens* test has been successfully used to assess toxicity in soils, contaminated ground waters and sediments. Acute toxicity

assayed with these methods is usually expressed as an EC_{50}–value, i.e. the xenobiotic concentration causing 50% inhibition of the luminescence.

Table 6. Bioluminescence based ecotoxicity test systems suitable for sediments, waters and soils and biomass analyses for sediments and soils

Test method	Measured parameter	Indicated toxicity	References
Vibrio fischeri	inhibition of luminescence	acute	ISO 11348, 1998
Pseudomonas fluorescens gen. modified	inhibition of luminescence	acute	APHA 1998
ATP	luminescence	acute	APHA 1998

ATP (adenosine 5'triphosphate) is present in all living cells. ATP disappears quickly after cell death. The amount of measured ATP can be used to quantitate live biomass (Table 6) (Alef and Nannipieri 1998, Sylvia et al. 1999). Contin et al. (2001) showed a strong correlation between the content of ATP and of biomass carbon from 207 samples of different soils ranging from acid to arable ($R^2 = 0.94$). ATP content has also been used to test for the toxicity of chemicals to forest soils (Bååth et al., 1991). A critical step in this analysis is the extraction of ATP from the sediment or from the soil (Sylvia et al. 1999, Alef and Nannipieri 1998). ATP is then measured from the extract by bioluminescence equation (2).



$$\text{(2) Reduced luciferin} + ATP + O_2 \xrightarrow{\textit{luciferase}} \text{oxidized luciferin} + CO_2 + ADP + P + \text{light } (h\nu)$$

Luciferin is an aromatic compound naturally present in fireflies. Luciferase enzyme catalyses the reaction of ATP with the reduced luciferin substrate and oxygen, so that light is emitted (Sylvia et al. 1998, Alef and Nannipieri, 1998). The amount of light produced is directly proportional to the amount of ATP. The light emission is measured with a luminometer. Luminescence based ATP measurement is a million fold more sensitive than spectrophotometric ATP measurements and 10^3 – 10^4 fold more sensitive than ATP measurements with synthetic chemiluminescence substrates such as luminol or lucigenin (Campbell 1988).

2. THE AIM OF THIS STUDY

The aim was study biodegradation activities in coniferous forest soils and freshwater sediments. More specifically the aims were following:

1. To assess the accumulation and the biodegradability of halogenated organic matter in sediments of lakes and rivers receiving or not receiving industrial discharges

2. Evaluate hydrolytic enzyme activities as tools for quantifying or describing biodegradation in soils and sediments

3. Analyse by a polyphasic approach the biodegradation of organic matter in sediments and soils.

4. Identify and describe factors affecting biodegradative microbial activities in soils and sediments.

5. Assess the vertical distribution and seasonal variation of biodegradative activities in the different layers of podsolized coniferous forest soils

6. Assess the contribution of cold seasons and the clear-cutting of tree stand on the biodegradative activities in podsolized coniferous forest soil

3. MATERIALS AND METHODS

3.1. Study sites

River Spittelwasser is located in the Bitterfeld region in south-eastern Germany. River Spittelwasser is tributary of river Mulde. The Bitterfeld region had large chlorine industry. Industrial and residential wastewaters were discharged to Spittelwasser. The area surrounding Spittelwasser in Bitterfeld is contaminated by organically bound chlorine (Götz et al. 1998). Sediment in the river Spittelwasser contained up to 120 ng I-TEQ of 2,3,7,8-TCDD / g dw (Bunge et al. 2001). The low flow speed favoured sedimentation in the River Spittelwasser (Bunge et al. 2001). The sampling site was in Spittelwasser river, which is in the midway (about 30 km) from the town of Halle to the town of Wittenberg. Details of the sampling site in Spittelwasser river is described in Bunge et al. (2001). The study areas of this thesis are listed in Table 7.

Vatavalkama and Tattari in the pulp mill recipient area are situated in south-eastern Lake Saimaa. The pristine forest Lake Pyylampi and the pristine forest Lake Mustalampi are situated about 20 km from the mill. Young *Pinus sylvestris* culture stand at Hyytiälä and an old naturally generated *Picea abies* stand at Mämmilampi are located in the vicinity of the Hyytiälä Forestry Field Station of Helsinki University.

Table 7. The study sites in this thesis

Study sites	Location	Described in
Hyytiälä, *Pinus sylvestris* stand	(61°48'N, 24°19'E)	Paper III, IV,V
Mämmilampi, *Picea abies* stand	(61°48'N, 24°19'E) 2 km from Hyytiälä pine forest	Paper III, IV, V
Vatavalkama basin in Lake Saimaa	(61°15'N, 28°49'E) 3 km from the mill	Paper I, II
Tattari basin in Lake Saimaa	(61°15'N, 28°49'E) 5 km from the mill	Paper I
Lake Pyylampi, pristine forest lake	(61°23'N, 28°24'E) 20 km from the mill	Paper I, II
Lake Mustalampi, pristine forest lake	(61°23'N, 28°24'E) 20 km from the mill	Paper I
River Spittelwasser in Bitterfeld in Germany	Sachsen-Anhalt between Halle and Wittenberg	This thesis

3.2. Methods in the study

Biodegradation activities in coniferous forest soils and freshwater sediments were investigated using methods shown in Table 8.

Table 8. Methods used in this thesis work.

Analysis	Description of method	Used in paper	Reference/ Manufacturer
Sampling of soil	Soil corer	III, IV, V	Westman 1995
Sampling of sediment	Freeze finger	I, II	Huttunen and Meriläinen 1978
Sampling of sediment	Limnos corer	I, II	Kansanen 1991
Sampling of sediment	Mondsee sampler (Giesbeek,The Netherlands)	this thesis	Bunge et al. 2001
Carbon and nitrogen content	Combustion followed by infra red detection	I, IV	Leco Corporation, St. Joseph, USA
Organic carbon content	Combustion followed by infra red detection	III, IV, V	Jokela and Salkinoja-Salonen 1992
Organic carbon content of Spittelwasser sediment	Combustion followed by infra red detection	this thesis	Rämisch 2000
Sediment EOX	Tetrahydrofuran extraction followed by combustion and microcoulometric titration of the halogen	I, II	Jokela and Salkinoja-Salonen 1992
Adsorbable organic halogen in sediment	Adsorption to active carbon followed by combustion and microcoulometric titration	this thesis	Rämisch 2000
Adsorbable organic halogen (AOX)	Adsorption to active carbon followed by combustion and microcoulometric titration	I	ISO standard 9562

Inorganic elements	Microwave digestion in nitric acid followed by ICP mass spectroscopy	I, II, V	Milestone, USA Fisons, Plasma Quard, Winsfont, U.K.
Hydrolytic enzyme activities	Kinetic fluorometry using fluorogenic substrates	II, IV, V, this thesis	Wittmann et al. 2000
Log K_{ow}-estimation	log K_{ow}-calculation program, log K_{ow}-version 1.66	V	Syracuse Research Corporation, Environmental Science Center, North Syracuse, N.Y., USA
Sediment acute toxicity	Inhibition of bioluminesence of Vibrio fischeri	II	ISO 1998
CO_2	Analysis of head space gas using gas chromatograph with thermal conductivity detector	III, IV	Schinner 1993
CH_4	Analysis of head space gas using gas chromatograph with thermal conductivity detector	III, IV	Wittmann et al. 2000
Soil pH	pH measurement of soil slurry	III, IV, V	Alef and Nannipieri 1998
Mineralization of ^{14}C-radiolabelled model xenobiotics	Microtiter plate radiorespirometry with phosphoimaging	IV	Fulthorpe et. al. 1996
In situ soil temperature	Thermocouples, Philips KTY81-110 and Nokeval 5020	III, IV	Haataja and Vesala (eds.) 1997
Precipitation	Tretjakov-type gauge	III, IV	Haataja and Vesala eds.1997
Soil water	Zero-tension lysimeter	III	Westman and Liski 1994
Nitrate and ammonium in precipitation and in soil water	Colorimetric	III	APHA 1998

4. RESULTS AND DISCUSSION

4.1. Accumulation and biodegradation of halogenated organic matter in sediments of lakes and rivers receiving or not receiving industrial waste waters

Stratification of halogenated organic matter was measured in lake and river sediments in eastern Finland and in south-eastern Germany. Figure 15 displays the stratification of organic bound halogen and Figure 16 that of organic carbon in the sediments of the Vatavalkama basin in Lake Saimaa (for location, see Figure 1 in Paper I), of the forest Lake Pyylampi at Ruokolahti (for the location, see Figure 1 in Paper I) and of the river Spittelwasser in Bitterfeld in Germany (location explained in Table 7). The sites were selected to represent areas with no (Lake Pyylampi) or heavy pollution (Vatavalkama in Lake Saimaa and River Spittelwasswer in Bitterfeld) by anthropogenic organic bound chlorine. Vatavalkama represented a site polluted by the pulp and paper industry. River Spittelwasser in Bitterfeld represented a site polluted by the chemical industry. Both sites were recipients of industrial discharges for over 50 years. The profiles of accumulated organic halogen in the three sediments show stratification (Figure 15). The sediment of forest Lake Pyylampi receiving no anthropogenic discharges displayed an in-depth gradient of tetrahydrofuran extractable organic halogen (EOX) from 20 mg of Cl m^{-2} (1 cm^{-1}) at the depth of 0 – 2 cm to 90 mg m^{-2} at the depth of 10 – 12 cm (Fig. 15B). The Lake Pyylampi sediment at the depth of 0 –2 cm contained organic carbon 1/5 of that at the depth of 10 – 12 cm (Figure 16). Suominen et al. (2001) showed that the molar ratio of Cl_{EOX}:C varied from 1:6000 to 1:14700 between the depths of 0 – 12 cm in Lake Pyylampi. The results of Suominen et al. (2001) and Figures 15A and 16A show that the halogen content of sediment organic matter was similar with depth in Lake Pyylampi.

The sediment accumulation rate of extractable organic halogen was 6 – 7 mg Cl m^{-2} $year^{-1}$ (Figure 3 in Paper I) in surface in the Lake Pyylampi. Jokela et al. (1992) reported that the organic halogen content of wet precipitation was 4.1. mg Cl m^{-2} $year^{-1}$ 15 km from the City of Imatra in the same area. To compare the sediment organic halogen content measured as tetrahydrofuran soluble halogen (EOX) to those of rain water (Jokela et al. 1992) measured as adsorbable organic chlorine (AOX), a conversion is needed. No such conversion factor is available for wet precipitation. Jokela and Salkinoja-Salonen (1992) showed that the EOX method used by us solubilized 80 – 97% of the AOX of untreated bleached kraft pulp mill waste waters. If EOX and AOX are closely similar also in the sediment and rainwater, the accumulation rate of EOX could be equivalent to 6 - 10 mg Cl m^{-2} $year^{-1}$ as AOX in Lake Pyylampi sediment. The annual atmospheric wet precipitation reported by Jokela et al. (1992) therefore indicates that the atmospheric fallout may directly contribute up to 43 – 65% of sediment accumulated organic halogen. The surface area of Lake Pyylampi is 0.063 km^2 and that of the catchment area is 3.2 km^2 (Paper I). The ratio of catchment area to surface area of Lake Pyylampi is 50. If all

organically bound halogen in the wet precipitation from the catchment area sedimented in Lake Pyylampi, then the sediment would have accumulated 20 – 30 fold more extractable organically bound halogen than was measured in the surface layer (0 – 12 cm) in Lake Pyylampi sediment. This calculation shows that all organic bound halogen in the wet precipitation from the catchment area did not accumulate in Lake Pyylampi sediment indirectly. A considerable amount of organic bound halogen compounds may have become degraded or are stored in the soil of the catchment area. Suominen et al. (2001) discussed the possible sources of organic halogen compounds in the Lake Pyylampi area and concluded that the organic bound halogen compounds in the sediment originated from the catchment area. We conclude here that a considerable part (over 40%) of organic halogen compounds, which sedimented to Lake Pyylampi came probably directly or indirectly from the atmospheric fallout as the runoff from the catchment area of Lake Pyylampi.

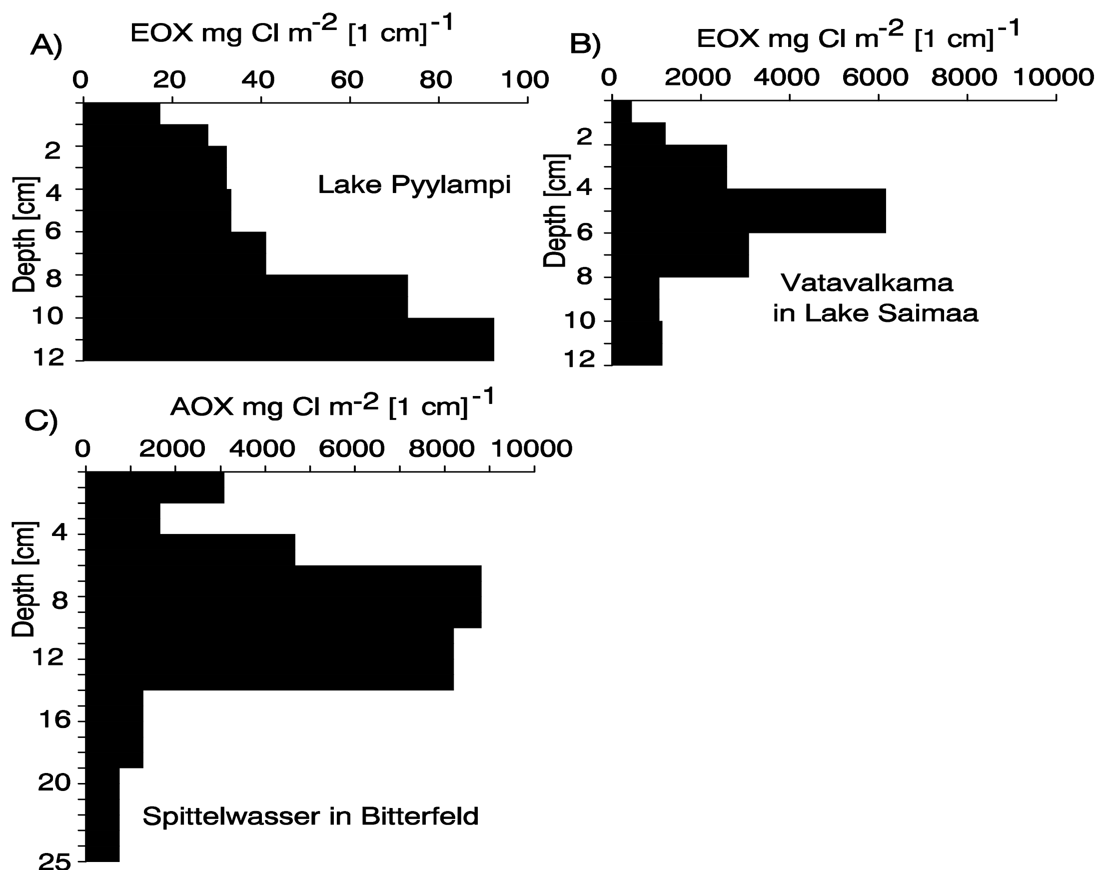


Figure 15. Vertical distribution of organic bound halogen in selected river and lake sediments. A. EOX (tetrahydrofuran extractable organic halogen) in the sediment of the forest Lake Pyylampi sediment receiving no direct anthropogenic discharges. Lake Pyylampi is situated at Ruokolahti 20 km NW from the pulp mill. B. EOX (tetrahydrofuran extractable organic halogen) in Vatavalkama sediment in pulp mill recipient area of Lake Saimaa. C. Adsorbable organic halogen (AOX) in the River Spittelwasser sediment in the Bitterfeld industrial region (Sachsen-Anhalt) in Germany. The sediment was sampled in October in 1999. Spittelwasser AOX data from Rämisch (2000).

Figure 15 B displays the content of extractable organic bound halogen in the pulp mill recipient sediment at Vatavalkama in Lake Saimaa. The content of extractable organic halogen (EOX) was highest (6.4 g Cl m^{-2} cm^{-1}) at the sediment depth of 4 – 6 cm at Vatavalkama. The content of accumulated extractable organically bound halogen was in all investigated sediment layers (0 – 12 cm) 20 – 200 fold higher than in those in the forest Lake Pyylampi located in the neighbourhood of the mill. This large difference between the organic bound halogen contents of Vatavalkama and Lake Pyylampi sediments was not reflected in the organic matter content (Fig. 16), which was only 1.0 – 1.7 fold higher at Vatavalkama than in the Lake Pyylampi sediment at the same depth (Figure 16). Suominen et al. (2001) showed that molar ratio of Cl_{EOX}:C 1:250 at the surface (0 – 2 cm) increased to 1:125 at the depth of 4 – 6 cm then decreased to 1:550 at the depth of 10 – 12 cm. The results of Suominen et al. (2001) and the results of Figures 15B and 16 B show that the overall quality of the sediment organic matter changed as a function of the depth from the surface.

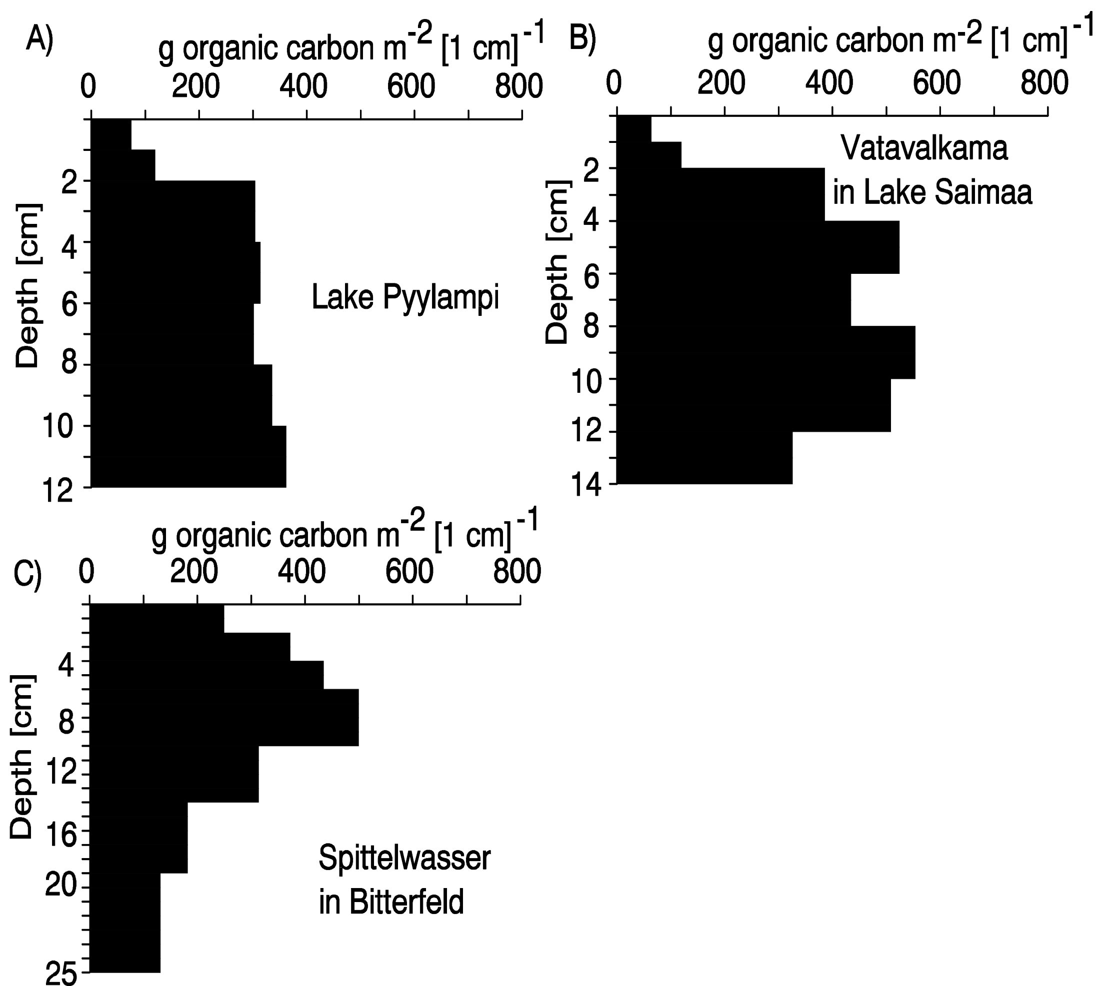


Figure 16. Vertical distributions of organic carbon in the sediments of A., Lake Pyylampi at Ruokolahti, B., Vatavalkama basin in Lake Saimaa and C., River Spittelwasser in Bitterfeld.

Figure 15C displays the adsorbable organically bound halogen (AOX) contents of the sediment of River Spittelwasser in the heavily industrialised Bitterfeld area in Germany. This sediment contained a layer (6 – 14 cm) where the organic bound halogen content (AOX) was high, (8.2 – 8.8 g Cl m^{-2} (1 cm^{-1}). Figures 15C and 16C show that the molar ratio of Cl_{AOX}:C was 1:83 at the depth of 6 – 10 cm and 1:56 at the depth of 10 – 14 cm and lower up to six fold of those in the above (0 – 6 cm) and below (14 –25 cm) situated sediment layers. The results in Figures 15C and 16C show that the organic halogen content of the organic matter changed with the depth of the sediment profile.

As indicated in p. 38, the EOX was 80 – 97% of the AOX in pulp mill waste water was extractable in tetrahydrofuran (Jokela and Salkinoja-Salonen, 1992). If AOX in the sediment is similarly extractable, the measured EOX content of the Vatavalkama sediment (4 – 6 cm) corresponds to 6.6 – 8.0 g of Cl m^{-2} cm^{-1} as AOX This is similar to that in the heaviest organic halogen (AOX) polluted layers (6 – 14 cm) in the sediment of River Spittelwasser in Bitterfeld, 8.2 – 8.8 g Cl m^{-2} cm^{-1}. The contents of organic bound halogen in the heaviest polluted sediment layers of River Spittelwasser at Bitterfeld and at Vatavalkama in Lake Saimaa were 60 - 400 fold higher than those in Lake Pyylampi. This shows that intensive and persistent sediment pollution by organically bound halogen has occurred in Spittelwasser in Bitterfeld and at Vatavalkama in Lake Saimaa in the past.

4.2. Heavy metals accumulated in the sediments receiving or not receiving industrial waste waters

We investigated if there was any relation between the contents of inorganic elements in lake sediments and pulp bleaching. Figure 17 displays the contents of 12 inorganic elements in sediments of the Vatavalkama basin in Lake Saimaa (receiving pulp industry waste waters) and in the forest Lake Pyylampi at Ruokolahti (receiving no local anthropogenic discharges). Cr, Cu, Hg, Mn, Ni, Zn and V accumulated to 2 to 8 fold higher contents in the pulp mill recipient area at Vatavalkama than in the nonrecipient forest Lake Pyylampi at the same sediment depth (Figure 17, see also Figure 2 in Paper II). This can be only partially explained by the sediment consistency as the carbon content at Vatavalkama was less than twofold that in Lake Pyylampi in the same depth. The Vatavalkama sediment contents of the 12 elements increased from surface (0-1 cm) by a factor of three to 2 – 8 cm and ninefold to the depth of 8 – 14 cm. The results in Figures 17 show contamination of Vatavalkama sediment with heavy metals at the depth of 8 – 14 cm. This can not be explained by sediment consistency as the carbon content of the layers from 2 – 4 to 12 – 14 cm was constant (Figure 16). The layers (Figure 15) with heaviest organically bound chlorine pollution (4 – 6 cm) was located above those that contained most of the heavy metals (12 – 14 cm) at Vatavalkama (Figure 17). This area of Lake Saimaa is close to the City of Imatra. The lower sediment content of Ni, V and Zn in the surface sediment (0 – 2 cm) compared to the layers below may relate to the change of fuel from coal and oil to natural

gas in this area in the 1980's. The fossil fuels contain Ni, V and Zn (Youngs 1993, Nriagu 1989, Nriagu and Pacyna 1988). The sediment contained Mn, Zn, Cu and Cr in the pulp mill recipient sediment at Vatavalkama could be related to timber floating and the debarking of logs, thus Mn, Zn, Cu and Cr belong to main heavy metals of wood as shown by analyses of wood ash by Moilanen and Issakainen (2000). Phenylmethyl mercury was used as a slimicide in the mills of Imatra area until 1967, when mercury containing slimicides were banned in Finland (Häsänen, 1975). The contents of mercury in Vatavalkama sediment was similar in the layers deposited before and after 1967, i.e. 6 - 8 cm (dated by Suominen 1999). This shows that the recipient lake sediment was not the main sink for the mercury discharged in waste waters. The highest sediment contents of the twelve inorganic elements analysed in Fig. 17 were detected in the deeper layers, from 8 to 14 cm. This is below the layers with the highest content of EOX (Figure 15), which was at the depth of 4 – 6 cm at Vatavalkama. These results show that the accumulation of toxic inorganic elements in the sediment at Vatavalkama was not related to pulp bleaching.

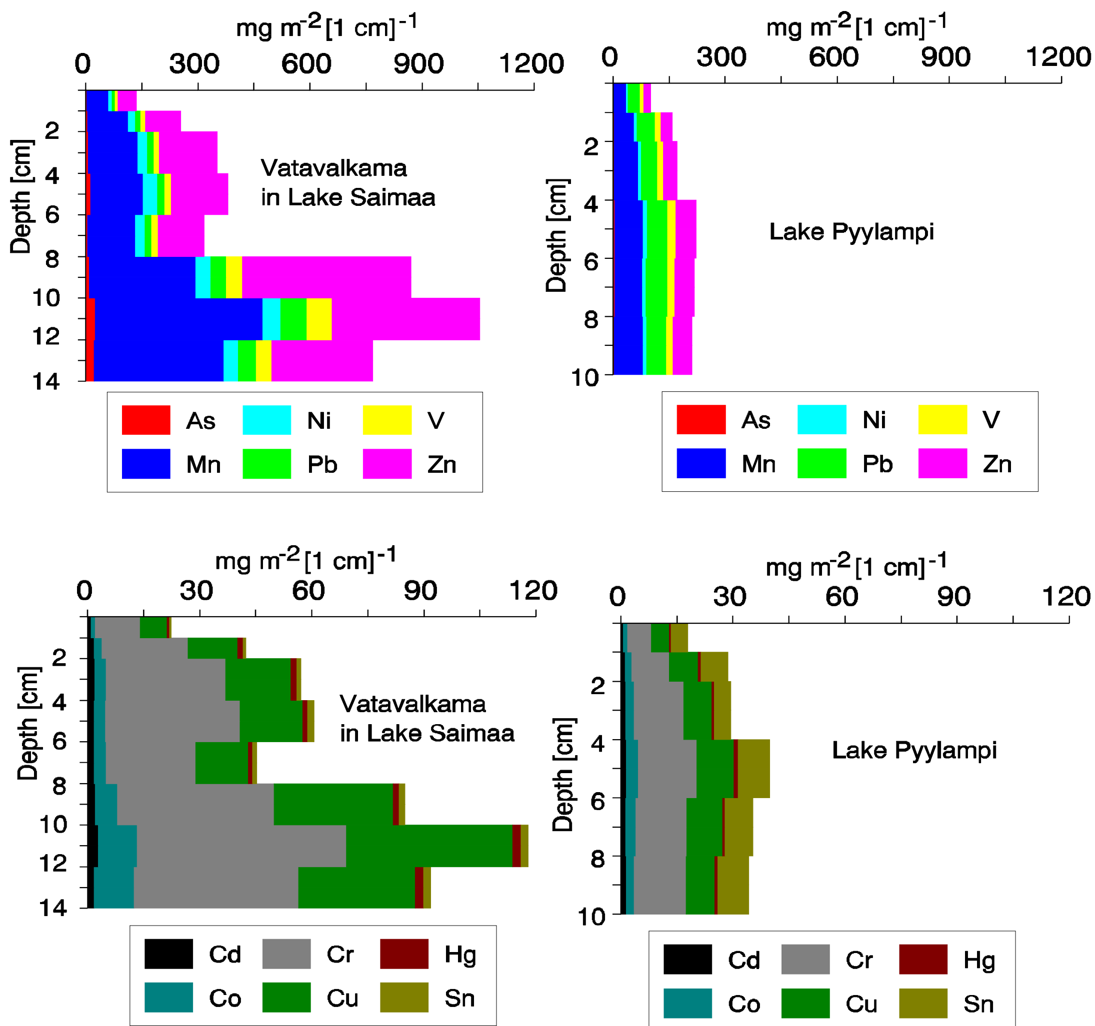


Figure 17. The contents of selected toxic inorganic elements in the pulp mill recipient sediment from the Vatavalkama basin, in Lake Saimaa and in the sediment of forest Lake Pyylampi receiving no direct anthropogenic discharges.

4.3. Polyphasic analysis of biopolymers degradation in soils and sediments

4.3.1. Hydrolytic enzyme activities related to microbial biomass

To assess the relation of sediment enzyme activities to the quantity of living microbial biomass, the activities of hydrolytic enzymes were compared to the sediment content of ATP. Figure 18 displays the results obtained for the sediments from Vatavalkama in Lake Saimaa and from Lake Pyylampi. The linear positive correlation was found for the activity of butyrate-esterase ($r = 0.89$, $p < 0.05$, $n = 6$) (Figure 18). The observed correlation indicates that the butyrate-esterase activity was related to the microbial biomass in a quantitative manner. The measured activities of butyrate-esterase were up to two orders of magnitude higher than those measured for aminopeptidase or for phosphomonoesterase. Thus butyrate-esterase seems to be a sensitive indicator for estimating the amount of live microbial biomass in sediments. log K_{ow} –value of MUF-butyrate is 3.4 (Table 9) indicating that this surrogate substrate is sufficiently lipophilic to diffuse passively through the microbial cell membrane. This means that the activities of both the extracellular and intracellular esterases will be measured when MUF-butyrate is used as a substrate. The high butyrate-esterase activity combined with lipophilicity of the substrate, MUF-butyrate, is good combination for assessing the quantity of microbial biomass. The activity of aminopeptidase also correlated positively ($r = 0.86$, $p < 0.05$, $n = 6$) to the sediment ATP content (Figure 18), but the slope in Figure 18 is too low for using the activity of aminopeptidase to quantitate microbial biomass.

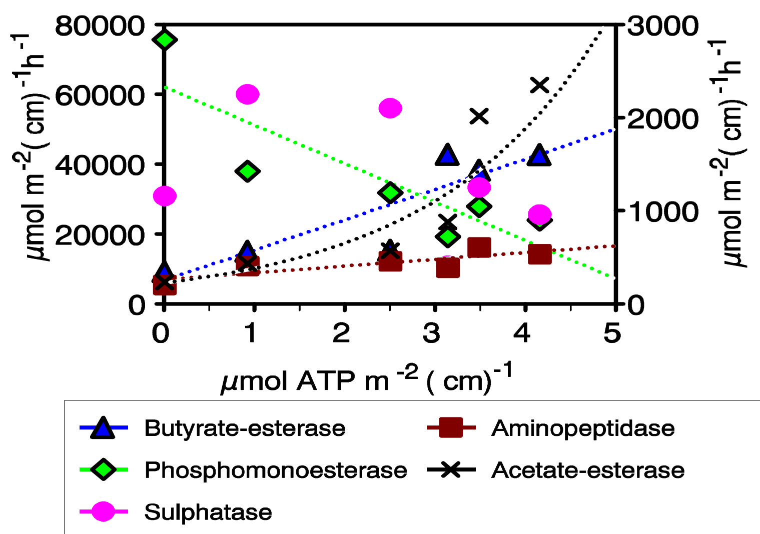


Figure 18. Activities (μmol m^{-2} (cm)$^{-1}$ h^{-1}) of phosphomonoesterase, aminopeptidase, sulphatase, acetate- and butyrate-esterase in relation to the content of ATP in the sediments from Vatavalkama in Lake Saimaa and from Lake Pyylampi. The activities of acetate- and butyrate-esterases are read from the left axis and those of sulphatase, aminopeptidase and phosphomonoesterase from the right axis. Activities of sulphatase were multiplied by a factor of 10 to be visible in the Figure.

Phosphomonoesterase activity had an apparent negative correlation with sediment ATP content ($r = -0.84$, $p < 0.05$, $n = 6$). Phosphomonoesterase activity was proposed by Sinsabaugh et al. (1991) as a tool to quantify microbial biomass in biofilms. Our results (Figure 18) show that it is not a useful indicator for lake sediments. The negative correlation between phosphomonoesterase activity and the ATP content in sediment seen in Figure 18 may relate to a low amount of available phosphate in the sediment. When the amount of phosphate is growth limiting, it leads to derepression of phosphomonoesterase activity (Saa et al. 1998). Dilly and Nannipieri (2001) showed that phoshomonoesterase activity in soil did not correlate with the soil ATP content, showing that phoshomonoesterase activity was not suitable for quantitating microbial biomass.

The activity of acetate-esterase showed an exponential positive relation to sediment ATP content ($r = 0.95$, Figure 18, $n = 6$). The exponential relationship between activity of acetate-esterase and sediment ATP content may be linked to the lower log K_{ow} –value of MUF-acetate of (2.3) compared to MUF-butyrate (3.4) (Table 9). MUF-acetate may be insufficiently lipophilic to saturate the microbial cell interior with the substrate of acetate-esterase. The hydrophilic (log K_{ow} –values < 0) substrates in Table 9 are too hydrophilic to passively diffuse into the microbial cells.

Table 9. The lipophilicities of the used fluorogenic 4-methylumbelliferyl (MUF) and 4-methyl-7-amino-coumarin (AMC) conjugated substrates expressed in their log K_{ow}-values. These values were calculated based on structural formulas using a software designed for log K_{ow}-calculation (Syracuse Research Corporation).

Fluorogenic substrate	g mol^{-1}	log K_{ow}	Substrate for enzyme
4-methylumbelliferyl phosphate	256.2	- 2.07	phosphomonoesterase
4-methylumbelliferyl sulphate	294.3	- 1.67	sulphatase
4-methylumbelliferyl butyrate	246.3	3.37	butyrate-esterase
4-methylumbelliferyl acetate	218.2	2.32	acetate-esterase
4-methylumbelliferyl-β-D-N-acetylglucosamide	379.4	- 0.35	N-acetyl-glucosamidase
4-methylumbelliferyl-β-cellobioside	500.5	- 2.20	β-cellobiosidase
4-methylumbelliferyl-α-D-glucoside	338.3	- 0.70	α-glucosidase
4-methylumbelliferyl-β-D-glucoside	338.3	- 0.70	β-glucosidase
4-methylumbelliferyl-β-D-xyloside	308.3	- 0.24	β-xylosidase
4-methyl-7-amino-coumaryl-leucine	324.8	- 0.60	aminopeptidase

4.3.2. Hydrolytic enzyme activities in lake and river sediment with reference to toxicity

The hydrolytic power of lake and river sediments contributing to the cycling of carbon, nitrogen, phosphorus and sulphur were measured. Figure 19 displays the vertical distributions of such activities in the sediments of the Vatavalkama basin, located in a pulp mill recipient area of Lake Saimaa, and of the forest Lake Pyylampi in a neighbouring region. Figure 20 displays the activities of the same enzymes in the sediment of River Spittelwasser at Bitterfeld (Sachsen-Anhalt) in Germany. The sediment activities (mmol m^{-2} cm^{-1} h^{-1}) of acetate-esterase, aminopeptidase, N-acetyl-glucosamidase, sulphatase, phoshomonoesterase and the carbohydratases, α-glucosidase, β-xylosidase and β-glucosidase were high in the oldest layers (10 – 12 cm), 20 – 30% of those in the surface sediment (0 – 2 cm). The organic matter content of the surface (0 – 2) layer (70 - 120 g C m^{-2} cm^{-1}) was only 1/3 to 1/5 of that in the below situated sediment layers (2 – 12 cm) in Lake Pyylampi (Figure 16) indicating that the organic matter in surface sediment was metabolically highly active. The activity of butyrate-esterase of Lake Pyylampi sediment showed no in-depth gradient, from the surface (0 – 2 cm) to the depth of 10 – 12 cm. This indicates that density of living biomass was constant over depths of 0 – 12 cm.

The activities of sulphatase, aminopeptidase, acetate- and butyrate-esterase in pulp mill recipient sediment at Vatavalkama at the depth of 2 – 6 cm were lower than those above or below. This indicates damaged microbiological functioning of the sediment at the depth of 2 – 6 cm, which was heavily contaminated with organically bound halogen, 2.6 – 6.2 g Cl of EOX m^{-2} cm^{-1}, (Figure 15). The ATP content of the Vatavalkama sediment (Figure 3 in paper II) was lowest (10 nmol ATP m^{-2} cm^{-1}) at the depth of 4 – 6 cm in the pulp mill recipient sediment. The low content of ATP and the low activity of butyrate-esterases at the depth of 2 – 6 cm indicate loss of microbial biomass (Figure 18) in these sediment layers, heavily contaminated with organically bound halogen (Figure 19). The layer at the depth of 4 – 6 cm was also 7 to 10 fold more toxic to *V. fischeri* (Figure 4 in paper II) than the layers below and above at Vatavalkama. The pulp mill discharging to the Vatavalkama area replaced elemental chlorine in the bleaching of pulp with chlorine dioxide and nonchlorine chemicals in bleaching of pulp from 1991 onwards. The mill waste waters were cleaned by an activated sludge plant that started full scale operation in the 1993. Archibald et al. (1998) showed that biologically treated pulp and paper mill effluents were not acutely toxic in the Microtox *Photobacterium* luminescence bioassay, although they were toxic to *Ceriodaphnia dubia* after chronic exposure. After the replacement of elemental chlorine with chlorine dioxide and nonchlorine chemicals and the start of the activated sludge plant to operate in full scale U-shaped activity curve of sulphatase, aminopeptidase, acetate- and butyrate-esterases in Vatavalkama sediment indicate that the microbial community had recovered in the surface of sediment (0 – 2 cm).

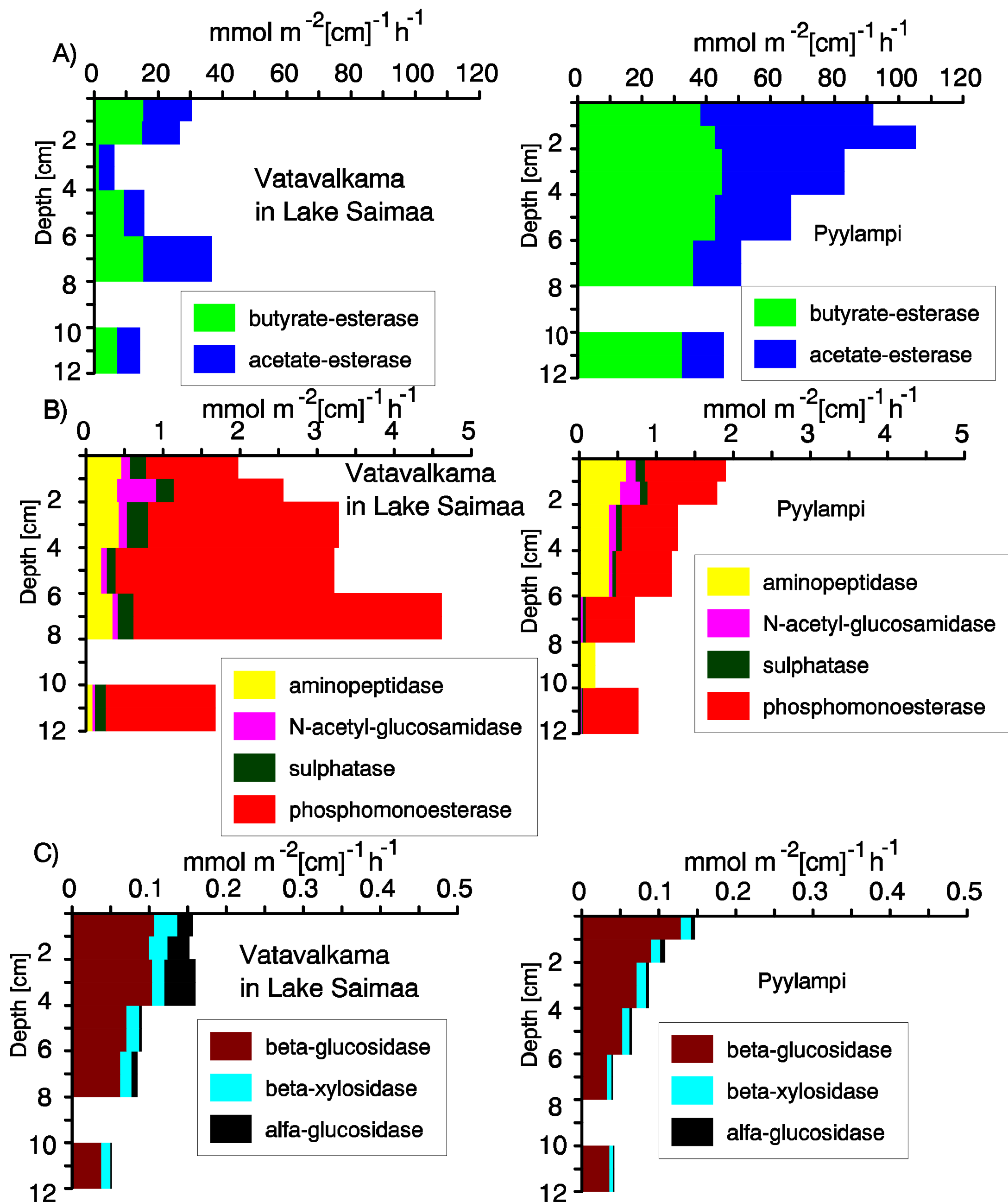


Figure 19. Vertical distributions of the potential (30 °C) activities of selected hydrolytic enzymes in sediments of the Vatavalkama pulp mill recipient basin in Lake Saimaa and the forest lake Pyylampi. Measured in July 1997. The substrates used for assessing the enzyme activities are shown in Table 9.

Phosphomonoesterase activity was higher in the Vatavalkama (Figure 19) sediment at the depth of 2 – 8 cm than above and below. The activity of phosphomonoesterase had a subsurface (2 – 8 cm) maximum at Vatavalkama in the sediment layers which were heaviest polluted with organic bound halogen indicating that phosphate was limiting in the heaviest polluted layers. Carbohydratases showed lowering activities from the surface towards the depth in the pulp mill recipient sediment in a similar manner to the nonrecipient forest Lake sediment Pyylampi in the same area (Figure 19). The activities of carbohydratases in the Vatavalkama sediment (0.05 – 0.16 mmol m^{-2} cm^{-1} h^{-1}) were close to those in the Lake Pyylampi sediment (Figure 19). The results in Figure 19 show that the activities of carbohydratases were not depressed even in the heaviest organic halogen (EOX), 6.2 g Cl m^{-2} (1 cm^{-1}), polluted sediment layers (2 – 6 cm) in the pulp mill recipient area at Vatavalkama. This indicates that carbohydratases activities were not sensitive to pollution by organic bound halogen indicating that the hydrolysis of cellulose and hemicellulose was not disturbed in the pulp mill recipient sediment.

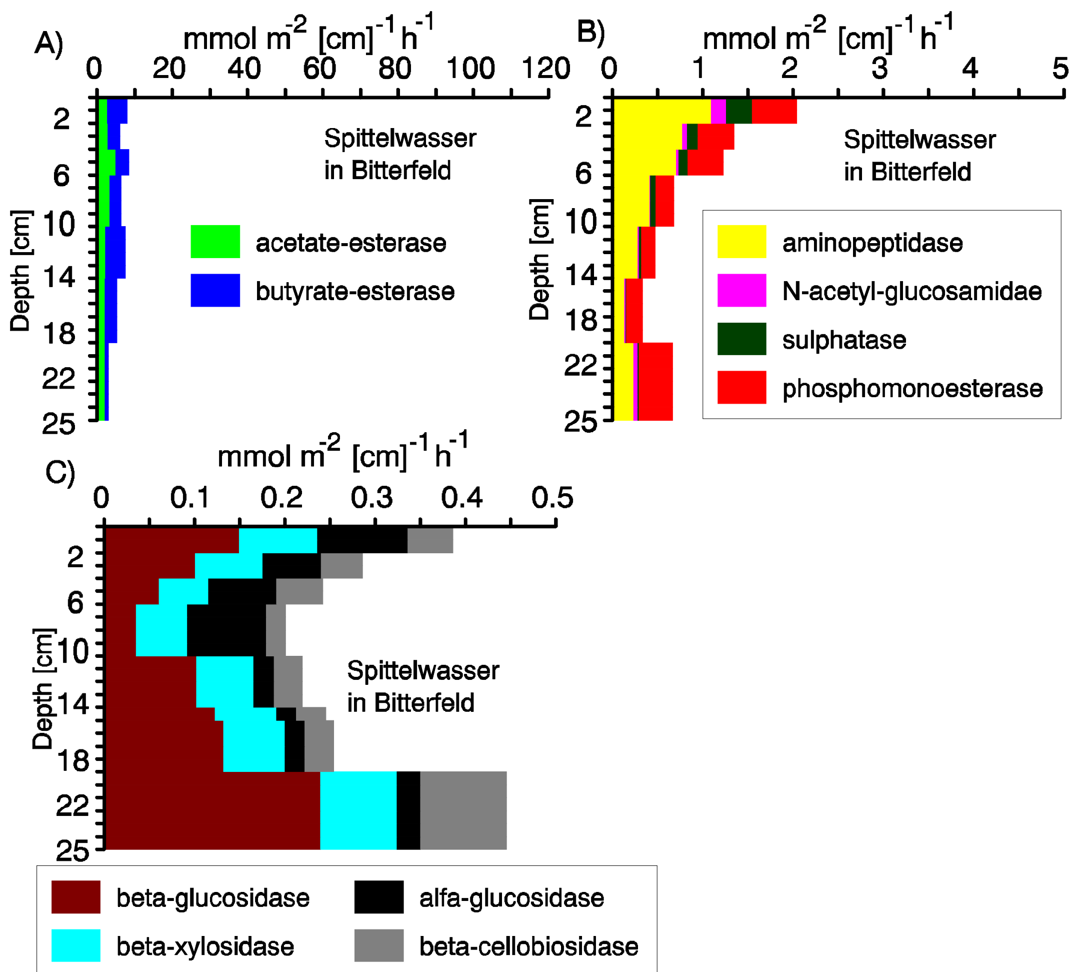


Figure 20. Vertical distributions of the potential (30 °C) activities of selected hydrolytic enzymes in the Spittelwasser river sediment in Bitterfeld. Sampled in October 1999 and measured on-site.

Figure 20 (A,B,C) displays the vertical distributions of activities of selected hydrolytic enzymes related to the cycling of phoshorus, nitrogen, sulphur and carbon, in the sediment layers of River Spittelwasser in Bitterfeld. The activity of butyrate-esterase in Spittelwasser sediment was 1/12 – 1/ 2 of those in Vatavalkama or in Pyylampi at the same depths excepting the depth of 2- 4 cm at Vatavalkama where esterase activity was also low (Figure 19A), close to that in Spittelwasser (Figure 20A). Considering the conclusion in chapter 4.3.1. that sediment butyrate-esterase activities are suitable for estimating the quantity of microbial biomass, the results in Figure 20 indicate that there was little living microbial biomass in River Spittelwasser sediment.

The River Spittelwasser sediment activities (mmol m^{-2} cm^{-1} h^{-1}) of β-glucosidase, β-cellobiosidase, sulphatase, aminopeptidase, N-acetyl-glucosamidase and phosphomonoesterase showed U-shaped activity curves with sediment depth (Figure 20). The activities were lower at the depth of 6-19 cm than above or below situated sediment layers. The result indicates damaged biodegradative activities in the heaviest organic chlorine polluted sediment layers (6 – 19 cm). Brack et al. (1999) reported that grab samples from 0 – 20 cm depth of the River Spittelwasser sediment were toxic to *Vibrio fischeri*, *Daphnia magna* and *Scenedesmus vacuolatus*. The U-shaped activity curves of β-glucosidase, β-cellobiosidase, sulphatase, aminopeptidase, N-acetyl-glucosamidase and phosphomonoesterase in Spittelwasser indicates recovery in the functioning of the surface sediment, although it contained organic bound chlorine 1/3 of that in the most polluted layers.

Figure 21 displays the Spittelwasser sediment activities of α-glucosidase, β-glucosidase, β-xylosidase, β-cellobiosidase, sulphatase, N-acetyl-glucosamidase, phosphomonoesterase, aminopeptidase, acetate- and butyrate-esterase expressed per g of organic carbon. Figure 22 displays the adsorbable organic halogen content per g of organic carbon in the same sediment layers. The activities of β-glucosidase, β-cellobiosidase, sulphatase, N-acetyl-glucosamidase, phosphomonoesterase and aminopeptidase were lower in the layers most polluted with organic halogen (35-53 mg Cl g of TOC^{-1}) located at the depth of 6 to 14 cm (Figure 22) than above or below indicating damage to the metabolic functioning of the sediment. This activity gap was not caused by due lack of sediment organic matter (Figure 16). Wittmann et al. (2000) showed that in the Vatavalkama sediment the activities related to cycling of sulphur, phosphorus and nitrogen were lower at the depth of 2 – 6 cm than the sediment layers situated above and below. In Spittelwasser the situation was similar, but in addition the β-glucosidase and β-cellobiosidase activities per g of organic carbon had the gap (Figure 21). Comparison of the results in Figure 22 A-C with those described by Wittmann et al. (2000) indicate that there was more severe damage in metabolic functioning of the polluted sediment layers in the Spittelwasser in Bitterfeld than that observed in the Vatavalkama sediment.

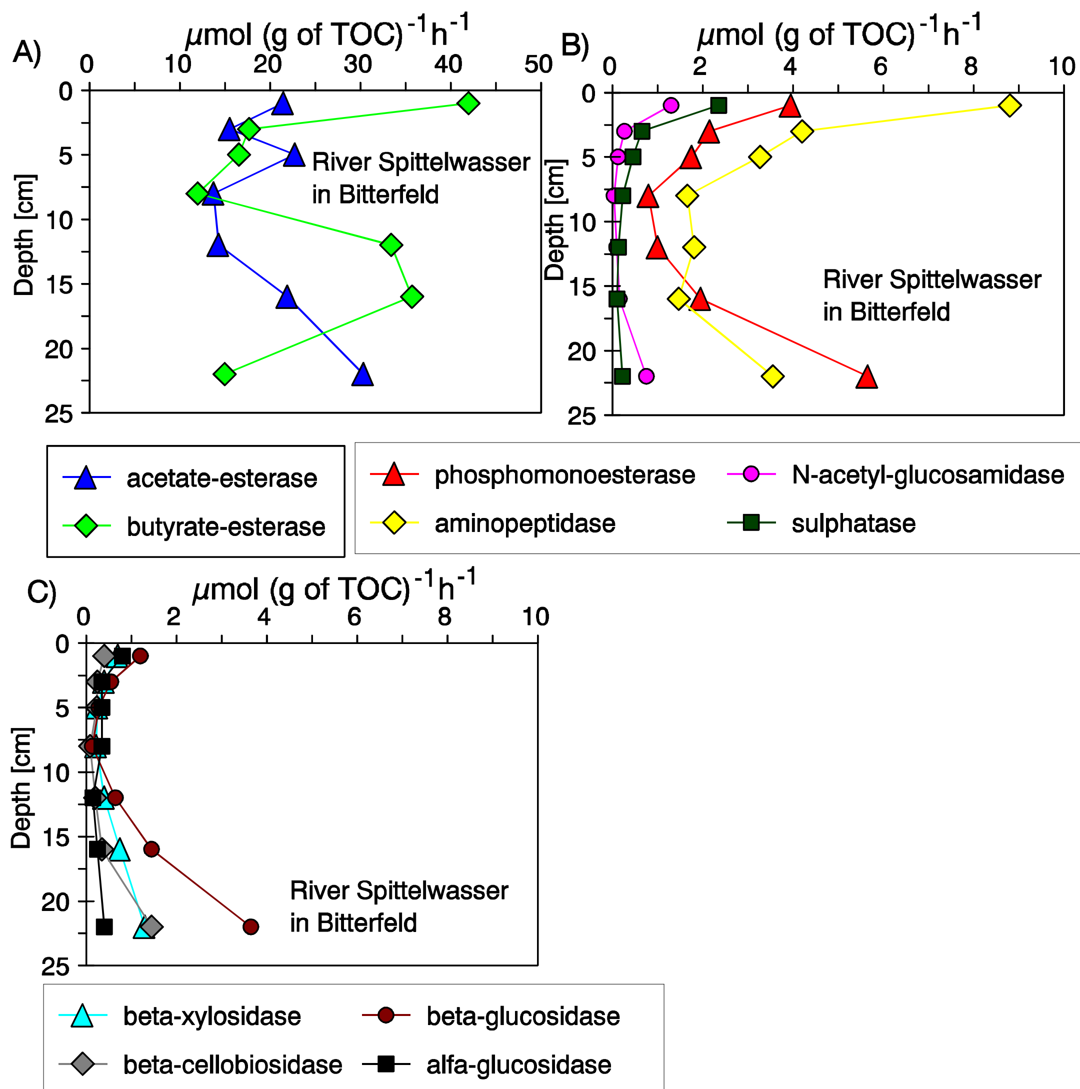


Figure 21 A-C. Vertical distributions of the organic carbon based potential activities (30 °C) of selected hydrolytic enzyme activities in Spittelwasser river sediment in the Bitterfeld region. Measured in October 1999.

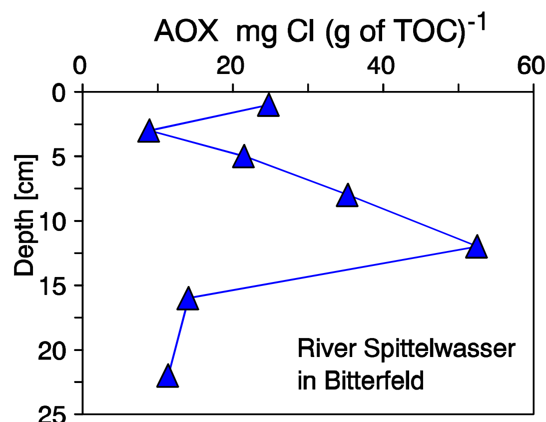


Figure 22. Vertical distribution of adsorbable organic halogen in sediment contained organic matter of Spittelwasser river in the Bitterfeld region. Data from Rämisch (2000).

4.3.3. Organic matter biodegradative activities in Finnish coniferous forest soils

Twelve bioactivities were assessed in Finnish acid coniferous forest soils: including carbon dioxide production, methane oxidation and ten hydrolytic enzymes, related to the cycling of carbon, nitrogen, phosphorus and sulphur. This was done in ten separate campaigns during two years in a young *Pinus sylvestris* stand at Hyytiälä and in a mature *Picea abies* stand at Mämmilampi. The aim was to reveal the stratification of and the responses to temperature, season and the harvesting of tree stands on these bioactivities in podzolized, acid forest soils.

Figure 23 displays the vertical distribution of the activities of the ten hydrolytic enzymes investigated in August 1999 of Hyytiälä and Mämmilampi forest soils. The activities of acetate-esterase, butyrate-esterase, β-glucosidase, β-xylosidase, α-glucosidase, β-cellobiosidase, N-acetylglucosamidase and phosphomonoesterase were high in the humus layer (0 – 4 cm) of both tree stands and decreased with soil depth. This was the case in all seasons over two years (Figures 2,3,4 in Paper V). The organic carbon content was highest in the humus layer (Table 1 in Paper III). Activities of acetate- and butyrate-esterases (Figure 23 A) were 5 to 10 fold higher than those of the N, P, S nutrient cycling enzymes (Figure 23 B) and the carbohydratases (Figure 23 C) in the corresponding depths of Hyytiälä and Mämmilampi forest soils. The high phosphomonoesterase activity in the humus layer may indicate that phosphate limited growth in the humus layer as this is known to cause derepression of phosphomonoesterase activity (Saa et al., 1998). Our results showed that hydrolytic enzyme activities, related to the cycling of carbon, were most active in the humus layer, where organic carbon content was highest.

The activities of sulphatase and aminopeptidase located mainly (50 – 70%) below the humus layer in Hyytiälä and Mämmilampi forest soils in most cases (Figure 3 in paper V). Staddon et al. (1998) reported a 20 fold higher activity of acid phosphomonoesterase and 8 fold higher

sulphatase activity in the humus layer compared to mineral soil in *Pinus banksiana* forest in Ontario in Canada. However, these authors measured the activities at a pH 1.5 units above that of the soil. Our data were obtained at the indigenous soil pH. The fact that sulphatase and aminopeptidase activities were located in layers deeper than in the humus layer in most cases (Figure 3 in Paper V) may relate to the fact that the pH of the soil was higher in the illuvial layer than in the humus layer (Table 1 in Paper III) and more closer to the pH optima of sulphatase and aminopeptidase (Figure 1 in Paper V).

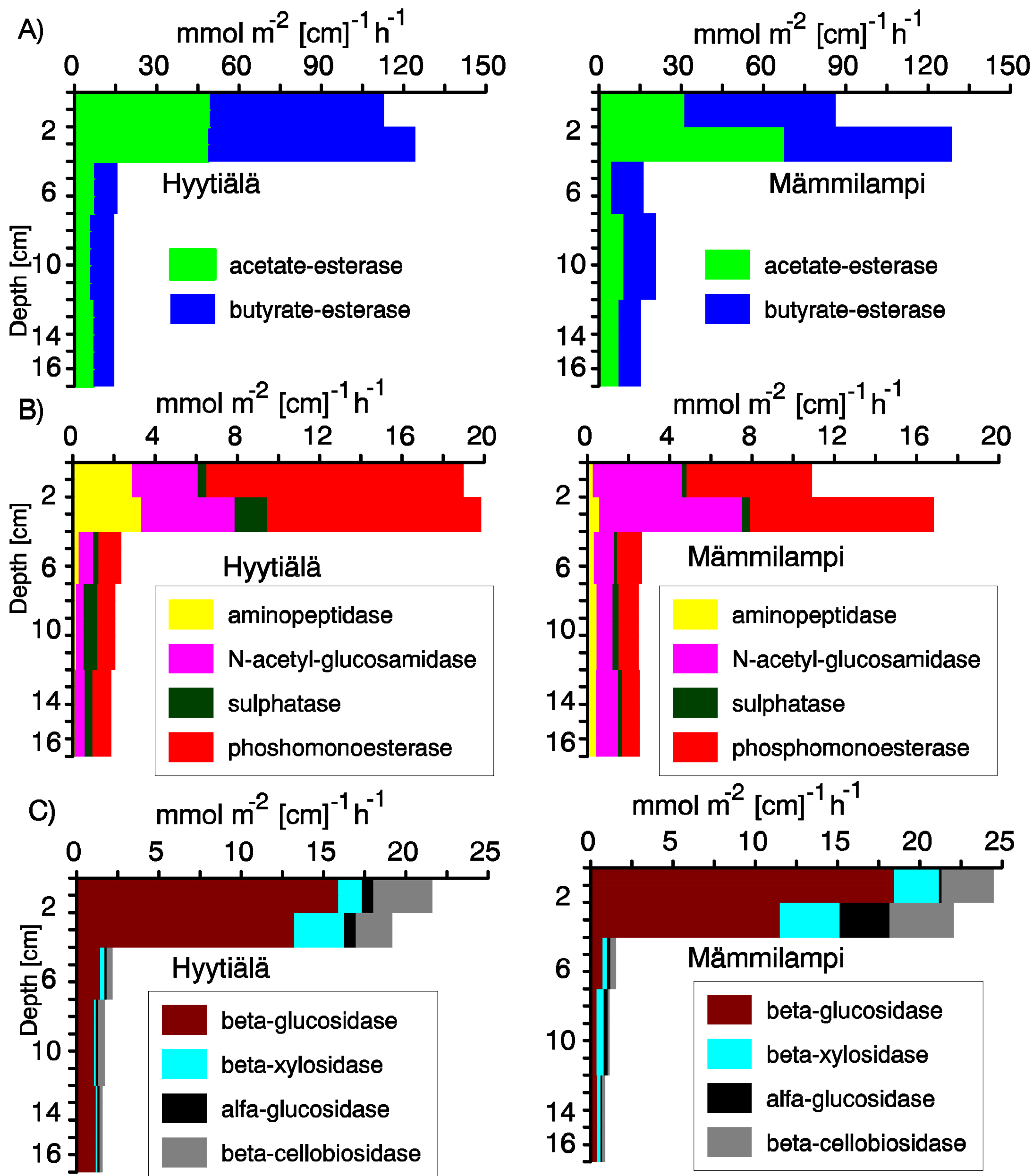


Figure 23. Vertical distributions of the potential (30 °C) activities of selected hydrolytic enzymes in the forest soils at Hyytiälä and Mämmilampi. Measured in August 1999 on site.

The activities of N-acetyl-glucosamidase, α-glucosidase, β-xylosidase, sulphatase, aminopeptidase, butyrate-esterase per soil organic matter (μmol h^{-1} g^{-1} soil organic carbon) were similar in the different layers in *Pinus sylvestris* forest soil at Hyytiälä (Figures 5,6,7 in Paper V).

In *Picea abies* forest soil the activities of α-glucosidase, β-xylosidase and β-cellobiosidase (μmol h^{-1} per g of soil organic carbon, Figure 5 in Paper III) were similar in the different layers. Sulphatase and aminopeptidase activities (Figure 6 in Paper V) increased towards the deeper layers in *Picea abies* forest. Table 10 combines hydrolytic enzyme activity results collected from the literature and calculated per g of soil organic carbon. Literature data compiled in Table 10 show that, when the organic matter content was taken into account, phosphomonoesterase, sulphatase, aminopeptidase, α-glucosidase and β-glucosidase activities were independent of the soil depth showing that hydrolytic enzyme activities were directly related to the amount of organic matter in the different depths of the studied soils.

Table 10. Hydrolytic enzyme activities calculated as μmol (g of TOC)$^{-1}$ h^{-1} from the literature data at the different depths of soils.

Activity	Site	Enzyme activity in different depths of soil μmol (g of TOC)$^{-1}$ h^{-1}		References
Phosphomonoesterase	*Eucalyptus diversicolor* forest in Australia	170 (0 - 2.5 cm)	230 (10 – 30)	Adams 1992
Sulphatase	Appalacian hill land in USA	40 in humus layer	58 in mineral soil	Balingar and Wright 1988
Aminopeptidase, α-glucosidase β-glucosidase	agricultural soil in USA	390 24 600 (0 –5 cm)	480 36 900 (10 – 15 cm)	Deng 1996, 1997
Aminopeptidase	Air dried sieved agricultural soil in USA	298 (0 – 5 cm)	269 (30 – 45 cm)	Frankenberger and Tabatabai 1981

The rates of carbon dioxide evolution were highest in the humus layer (0 – 4 cm) in *Pinus sylvestris* (Hyytiälä) and *Picea abies* (Mämmilampi) forest soils in all seasons during two years (Figure 1 in Paper III). The carbon dioxide evolution measured by us contains the carbon from the mineralization of carbon compounds and may contain carbon dioxide evolved from the fresh

root litter and root respiration, because it is possible that the broken roots continued to respire for a while. This is a natural component of carbon dioxide emission from the soils. Leiros et al. (1999) found that carbon dioxide evolution was 4.5 – 4.7 fold higher in the humus than in the below situated A-layer also in two Spanish Oak forest soils, where the total carbon content of the humus was 2.4 – 2.6 fold higher than that of the A-layer. Smolander and Mälkönen (1994) showed that the extractable microbial biomass carbon content, expressed on a soil volume basis, was in the humus layer 2 – 4 fold higher than in the eluvial and 9 – 14 fold higher than in illuvial layer in limed and control *Picea abies* stands in southern and central Finland. Fritze et al. (2000) showed that the microbial biomass, measured as SIR, decreased from humus to eluvial, illuvial and parent material at Mämmilampi in September 1994. The phospholipid fatty acid pattern differed between the humus, the eluvial and the upper and lower parts of the illuvial layer at Mämmilampi in September 1994 indicating a change in the microbial community structure with increasing depth (Fritze et al. 2000). Pietikäinen et al. (1999) showed the average microbial biomass carbon, measured as SIR, as a percentage of soil total organic carbon from May 1994 to September 1994 in humus (L+F+H), eluvial, illuvial and ground layers at Mämmilampi were 1.4, 1.5, 3.6 and 4.1%, respectively. Pietikäinen et al. 1999 concluded that microbes used new root litter and exudates from living roots in the layers situated below the humus layer. We showed that carbon dioxide production per soil organic carbon was similar in the lower part of the illuvial layer and the humus layer at Hyytiälä and Mämmilampi indicating a similar bioavailability of the soil carbon in both layers (Figure 5 in Paper III). Our carbon dioxide production results (Figure 1 and 5 in Paper III) and the results of Fritze et al. (2000), Leiros et al. (1999), Pietikäinen et al. (1999), Smolander and Mälkönen (1994) indicate that carbon dioxide production was related to the amount of organic matter and microbial biomass in the different layers of forest soil.

The activities of β-glucosidase, α-glucosidase, β-xylosidase, sulphatase, β-cellobiosidase, phosphomonoesterase, aminopeptidase, N-acetyl-glucosamidase, acetate- and butyrate-esterase (mmol m^{-2} h^{-1}) were higher or equal in October than in July-August over two years (Figure 2,3,4 in Paper V). Moreover, carbon dioxide evolution tended to be higher 2 – 30% higher in October than in July in *Pinus sylvestris* (Hyytiälä) and *Picea abies* forests (Mämmilampi) over two years (Figure 1 in Paper III). Dilly and Munch (1996) showed that β-glucosidase activities decreased to one-third after the first 30 days of decomposition of black alder leaf litter indicating that easily degradable organic matter had become utilized. Linkins et al. (1990) showed that β-glucosidase activities were threefold higher during the first 30 days in decomposing leaves of *Quercus prinus* and *Acer rubrum* than during the later stages indicating the higher availability of nutrients in the initial stages of decomposition. Whalen and Warnan (1996) showed that sulphatase activity was twofold higher in autumn than in spring in the agricultural soil (0 – 15 cm). Abdullah et al. (1996) showed that addition of sugar beet to agricultural soil as an easily available nutrient source, resulted in increased activities of amylase, sulphatase, phosphomonoesterase and urease in the soil. Our results from coniferous forests (Figure 1 in Paper III, Figure 2,3,4 in Paper V) and those from field soil (Whalen and Warnan 1996) and from litter (Linkins et al. 1990, Dilly and Munch 1996) indicate that bioactivities were regulated by other factors than soil temperature.

Methane consuming potential in the *Pinus sylvestris* forest soil at Hyytiälä and the *Picea abies* forest soil at Mämmilampi was mainly located in the illuvial layer in all seasons (Figure 1 in Paper III). Saari et al. (1998) reported that methane oxidation potential was mainly located at the depth of 11 – 16 cm in a podzolized *Pinus sylvestris* stand in Suonenjoki in Finland in July (*in situ* pH = 4.1) at 15 °C. Saari and Martikainen (2001) showed that methane was oxidized by methanotrophic bacteria, not by nitrifiers, in *Pinus sylvestris* stand in Suonenjoki, when dimethyl sulfoxide inhibited methane oxidation, but not nitrification. Schnell and King (1994) reported that the subsurface maximum of methane oxidation occurred at the depth of 4 – 8 cm (*in situ* pH = 4) in a mixed hardwood-conifer forest in Maine USA, when measured at 22 °C and 2 ppm methane. Since ammonia oxidation mainly located in the surface of soil (0 – 2 cm), Schnell and King (1994) concluded that the methane oxidation at 4 – 8 cm was not catalysed by ammonia-oxidizing bacteria. Prieme and Christensen (1997) reported that methane oxidation mainly located at the depth of 5 – 10 cm (*in situ* pH = 4.2) below the humus layer in an oak forest in Denmark and that an addition of 0.2 – 0.6 NH_4^+-N or NO^-_3-N dm^{-3} (soil) depressed this activity by 50 – 60%. Sundh et al. (2000b) reported for a *Picea abies* stand in middle Sweden that methane oxidation was mainly located at the depth of 10 – 15 cm (*in situ* pH = 4.2). Amaral et al. (1997) showed that aqueous extracts prepared from conifer forest humus (0 – 5 cm) suppressed methane oxidation by up to 100% in the methane oxidating layer of coniferous forest soil. Extracts prepared from deeper layers of the same forest soil did not inhibit methane oxidation. If such a similar inhibition by humus extracts occurs also in Hyytiälä and Mämmilampi forest soils, this could explain the location of the maximum of methane oxidation in Hyytiälä and Mämmilampi forest soils below the humus layer as discussed in Paper III. Our results obtained from Hyytiälä and Mämmilampi forest soils show that in Finnish coniferous forest soil the illuvial layer is more favourable to methane oxidation than layers above. This may be due to factors such as more favourable soil pH (4.8 – 5.3) in the illuvial layer than the layers above and the absence of inhibitors e.g. ammonium, nitrate and humic substances as inhibitors or better access for soluble iron (Paper III).

4.3.4. Temperature response of detritus biodegrading activities in Finnish coniferous forest soils

Temperature dependence of endogenous carbon dioxide evolution, the potential for methane oxidation and organic matter hydrolysing enzyme activities were measured in the soil of a coniferous stand at Hyytiälä. The Q_{10}-values of endogenous carbon dioxide evolution measured for the humus layer in a Hyytiälä *Pinus sylvestris* stand ranged from 2.3 to 2.8 (Table 3 in Paper IV) at temperatures of -3 to + 12 °C indicating a moderate temperature dependence. The corresponding energies of activation (E_a) for carbon dioxide evolution were from 60 to 80 kJ mol^{-1} (Table 3 in Paper IV). Boone et al. (1998) reported similar Q_{10}-values, 2.3 – 4.5, for the endogenous evolution of carbon dioxide in a mixed-hardwood forest soil in temperate climate in Massachusetts, USA. Kirschbaum (1995, 2000) combined literature data of Q_{10}-values measured for various forest soils and forest litter from southern to middle Europe. Based on these, he

estimated that a typical Q_{10}-value at 0 °C was 8, at 10 °C 4.5 and at 20 °C 2.5. The Q_{10}-value estimated by Kirschbaum for carbon dioxide evolution thus decreased with increasing temperature. His estimation of Q_{10} of 4.5 – 8 for temperatures of 0 to 10 °C differs from Q_{10}-values of 2.3 to 2.8 (Table 3 in Paper IV) obtained by us for Hyytiälä forest soil at temperatures from –3 to 12 °C. The annual mean forest soil temperature in Hyytiälä is 5 °C (Figure 2 in Paper III). If our estimation of Q_{10} is valid for coniferous forest soils also elsewhere in the boreal zone, an increase of 1 °C of soil temperature would accelerate this evolution of carbon dioxide by 13% to 18% and not 35% - 70% as prognosed by the model of Kirschbaum (1995, 2000).

We also measured the Q_{10}-value for methane oxidation potential in the illuvial layer of Hyytiälä forest soil. It was 2.1 for the temperature range of 2 °C to 12 ° C indicating a moderate temperature dependence of methane oxidation. Bowden et al. (1998) reported a Q_{10}-value of 1.1 for methane oxidation in hardwood forest soil in Massachusetts in the USA, obtained by laboratory incubations at 5 °C to 15 °C. Prieme and Christensen (1997) reported a Q_{10}-value of 1.4 for methane oxidation in a Danish spruce stand in the field as well as in the laboratory (- 2 °C to 20 ° C) using soil cores. Our Q_{10}-values (Table 3 in Paper III) and those of Bowden et al. (1998) and Prieme and Christensen (1997) for methane oxidation are lower than the Q_{10}-values estimated for the endogenous evolution of carbon dioxide (2.0 – 2.8) in the same soils measured by the same groups indicating the involvement of other factors than temperature to limit the rate of methane oxidation.

Q_{10}-values ranging from 1.6 to 2.1 were found for the activities of β-glucosidase, acetate-esterase and butyrate-esterase in Hyytiälä forest soil at the temperature range from 14 to 30 °C (Table 4 in Paper IV). Parman and Deng (2000) reported Q_{10}-values of 1.4 – 2.5 for N-acetyl-glucosamidase (10 to 60 °C) in agricultural wheat soil in Oklahoma, USA. Acosta-Martiez and Tabatabai (2002) reported for aminopeptidase Q_{10}-values of 1.3 – 1.6 at 30 to 40 °C in unfertilised agricultural soil. Our Q_{10}-values, 1.6 – 2.1, measured for hydrolytic enzyme activities in boreal forest soil at 14 °C to 30 °C, were similar to those measured by Parman and Deng (2000) and of Acosta-Martiez and Tabatabai (2002) showing a rather weak temperature dependence of hydrolytic enzyme activities in the different types of soils. This could indicate that the hydrolytic exoenzyme potential is a limiting factor in litter turnover. Our results, however, showed that the forest soils at Hyytiälä and Mämmilampi had the potential to release > 1000 mols of ester linked carbon m^{-2}, > 700 mols of carbohydrate-carbon m^{-2}, > l00 mols of inorganic phosphate m^{-2} and 9 to 38 mols of inorganic sulphate m^{-2} (Tables 3,4,5 in Paper V). The annual above ground litter fall was 7 – 10 mol litter bound carbon m^{-2} (Paper III). The results show that the hydrolytic exoenzyme activities had a high excess capacity to hydrolyse the biogenic polymers deposited annually onto the forest as above ground litter fall indicating that this step is not likely to be rate limiting in litter turnover.

The Q_{10}-values measured for carbon dioxide evolution, potential for methane oxidation and for hydrolytic activities in Hyytiälä and Mämmilampi soils were used to calculate the *in situ* activities at actual soil temperatures (Table 2 in Paper III, Tables 3, 4, 5 in Paper V, Table 11). According to Markkanen et al. (2001), the growing season in Hyytiälä *Pinus sylvestris* soil

started on April the 28th in 1997, on April the 16th in 1998, on March the 25th in 1999 and ended on November the 1st in 1997, on November the 30th November in 1998 and on November the 14th in 1999. Based on this, we divided the year in the cold (Nov – Apr) and warm season (May – Oct). The *Pinus sylvestris* forest did not take up considerably carbon dioxide outside the growing period (Markkanen et al. 2001). Therefore all emitted carbon dioxide from the *Pinus sylvestris* soil was released into the atmosphere. The Hyytiälä area during winter time belongs to the thick snow and eastern frost area, but Hyytiälä area is close to the borderline, which divides Finland into a western thick frost area and an eastern frost area (Figure 14; Solantie, 2000). The snow cover protects the soils against penetration of frost. Markkanen et al. (2001) showed that the average net carbon dioxide emission rate was 0.44 μmol m^{-2} s^{-1} during the non-growing period in 1997 / 1998 and also in 1998 / 1999. This value is based on Eddy covariance measurements in *Pinus sylvestris* forest above the tree stand of Hyytiälä. Summed over the six month from November to April, 83 g CO_2-C m^{-2} was emitted. In the same six months our results showed that the carbon dioxide production represented 19% (81 g C m^{-2}) of the annual carbon dioxide evolution calculated to *in situ* temperature in the Hyytiälä forest soil column (17 cm) in 1997 / 1998 and 22% (98 g C m^{-2}) in 1998 / 1999 (Table 11, Table 2 in Paper III). The results (Table 11, Table 2 in Paper III) for Mämmilampi were 21% (132 g C m^{-2}) and 23% (149 g C m^{-2}) (Table 11, Table 2 in Paper III). Our results (Table 11, Table 2 in Paper III) were close to that measured as the carbon dioxide emission for whole *Pinus sylvestris* forest at Hyytiälä from the Eddy covariance data of Markkanen et al. (2001). The match between our soil emission results (Table 11, Table 2 in Paper III) and the forest emission (Markkanen et al. 2001) from Hyytiälä shows that, in the Hyytiälä *Pinus sylvestris* stand, the soil was the principal source of carbon dioxide from the forest into the atmosphere during the cold half of the year. McDowell et al. (2000) reported that 17% (132 g C m^{-2}) of annual cumulative carbon dioxide evolution measured using an *in situ* chamber during the snow covered period represented 34% of the annual emission from forest soil in Idaho, USA.

The capacity of Hyytiälä forest soil for methane turnover was in 1997 / 1998 24% of the annual and in 1998 / 1999 10% during the cold half of the year (Table 11). The corresponding figures for Mämmilampi were 21% and 13% (Table 11). The winter of 1998 – 1999 was colder (the mean air temperature from Dec to Feb was – 5.4 °C) than that of 1997 –1998 (the mean air temperature was – 2.9 °C) (Table 1 in Paper V). The results in Table 11 show that methane oxidation, but not carbon dioxide evolution, was suppressed by the lower temperature in winter 1998 / 1999 compared to winter 1997 / 1998. Prieme and Christensen (1997) showed that methane was oxidized in soil cores incubated in the laboratory at – 2 °C and King and Adamsen (1992) showed the same at –1 °C. Our results confirm those by McDowell (2000), by King and Adamsen (1992) and by Prieme and Christensen (1997), that methane oxidation and endogenous carbon dioxide occur at cold temperature. Our results show (Table 11, Table 2 in Paper III) that a considerable part of annual methane oxidation (10 – 24%) and carbon dioxide (19 – 23%) took place during the cold half of the year, although methane oxidation was more cold sensitive than total carbon dioxide evolution.

Table 11. Contribution of the non-growing season, from November to April, to the annual cumulative carbon dioxide evolution, the hydrolytic activity and the methane oxidation potential in the *Pinus sylvestris* forest soil at Hyytiälä and the *Picea abies* forest soil at Mämmilampi. All activities were calculated to *in situ* soil temperatures (Figure 2 in Paper III) measured on line in each layer throughout the year.

Contribution of the cold season (November – April) to the annual cumulative activities					
	in *Pinus sylvestris* soil		in *Picea abies* soil		Data from
	1997/8	1998/9	1997/8	1998/9	
Carbon dioxide evolution	19 %	22 %	21 %	23 %	Table 2 in Paper III
Methane oxidation potential	24 %	10 %	21 %	13 %	Table 2 in Paper III
Carbohydratases	7 - 19 %	12 – 17 %	9 – 18 %	16 – 22 %	Table 3 in Paper V
Hydrolytic activities related to nutrient cycling of P, N, S, C	21 – 32 %	13 – 32 %	13 – 24 %	9 – 16 %	Table 4 in Paper V
Esterases	nd	12 – 21 %	nd	17 – 18 %	Table 5 in Paper V
Mean air temperature [°C] (November – April)	- 2.4	- 3.2			
Soil column mean [°C] (humus, eluvial, illuvial) (November – April)	0.7	0.4	0.1	- 0.2	

nd = not determined

The carbohydratase activity potential was calculated for the measured (Figure 2 in Paper III) *in situ* temperature. The activity sum of these enzymes from November to April represented 7 to 19% in 1997 / 1998 and 12 – 17% in 1998 / 1999 of the total annual activity potential in Hyytiälä *Pinus sylvestris* forest soil (Table 11). The corresponding figures for Mämmilampi were 9 – 18% in 1997 / 1998 to 16 – 22% in 1998 / 1999 (Table 11). The hydrolytic activities, related to the nutrient cycling of phosphorus, nitrogen, sulphur and carbon represented 13 – 32% of the annual activity in Hyytiälä forest soils in the winter 1998 / 1999 and 21 – 32% in 1998 / 1997 (Table 11). The corresponding figures at Mämmilampi were 9 – 16% and 13 – 24% (Table 11). The good preservation of the soil organic matter and litter hydrolysing and mineralising activities outside the growing period (November to April) may depend on preservation by the enzymes as complexes with the humus or mineral soil, in a similar way to that observed in other types of soils by Neal (1990), Nannipieri et al. (1988), Rao et al. (1996) and Gianfera et al. (1994).

4.3.5. The cumulative annual carbon dioxide evolution in coniferous forest soils

The production of carbon dioxide at *in situ* temperature in the *Pinus sylvestris* (Hyytiälä) and *Picea abies* (Mämmilampi) forest soils was calculated by us to be 36 – 54 mol m^{-2} $year^{-1}$ between May 1997 and April 1999 (Table 2 in Paper III). McDowell (2000) reported that carbon dioxide evolution was 65 mol m^{-2} $year^{-1}$ *in situ* chamber measurements in coniferous forest soils, where snow pack time was 36% of the year. Rayment and Jarvis (2000) used the same method and found an emission rate of 75 mol of carbon dioxide m^{-2} $year^{-1}$ in a 115 year-old boreal black spruce forest in Canada. Our results (Table 2 in Paper III) were close to those reported by McDowell et al. (2000) and by Rayment and Jarvis (2000) indicating that snow covered coniferous forest soils produced similar quantities of carbon dioxide on two continents and that forest soils are important sources of carbon dioxide.

The potential for methane oxidation of Hyytiälä and Mämmilampi forest soils calculated to *in situ* soil temperature ranged from 0.16 to 0.33 mol m^{-2} $year^{-1}$ from May 1997 to April 1999 in the soil column (17 cm) (Table 2 in Paper III). This result means that methane oxidation must have contributed less than 1% to the endogenously evolved carbon dioxide (Table 2 in Paper III). The annual carbon dioxide evolution from soil, including mineralization of soil organic matter and possibly also root respiration as well as mineralization of fresh root litter, at Hyytiälä and Mämmilampi (36 – 54 mol m^{-2}, Table 2 in Paper III) was three to five fold higher than the carbon amount of the annual above ground litter fall on the same sites. Therefore root exudates, old aboveground litter, recent and old below ground litter must have served as substrates for a major part of the endogenously evolved carbon dioxide.

4.3.6. The impact of the clear-cutting of the tree stand on the biodegradative activities in forest soil

Mämmilampi *Picea abies* stand was harvested in March 1998. The activities of carbon dioxide evolution, methane oxidation potential and ten hydrolytic enzyme activities were compared between the year before and the year after the clear-cut (Table 2 in Paper III, Tables 3,4 and 5 in Paper V). Table 12 compares the changes in carbon dioxide evolution and in the potential activities of methane oxidation and of sulphatase in Hyytiälä and Mämmilampi soils in the year before (1997 / 1998) and after (1998 / 1999) the clear-cutting of the Mämmilampi tree stand. Nine of the measured enzyme activities, aminopeptidase, N-acetyl-glucosamidase, phoshomonoesterase, β-glucosidase, β-xylosidase, α-glucosidase, β-cellobiosidase, acetate- and butyrate-esterase followed similar trends in both years at Hyytiälä and Mämmilampi (Tables 3, 4, 5 in Paper V) indicating that the clear-cutting had no impact on these activities.

After the clear-cutting the endogenous carbon dioxide evolution from Mämmilampi forest soil at the *in situ* temperature diminished in the eluvial and illuvial layers and increased in the humus layer (Table 12). No such change was at Hyytiälä. The observed decrease of the endogenous

carbon dioxide evolution in the eluvial and illuvial layers, 160 g C m^{-2} $year^{-1}$ (Table 2 in Paper III) may have resulted from a decreased substrate delivery from direct root exudates (photosynthate), from the below ground litter from the annual fine rooth death and mycorrhiza to those soil layers. The results of Ohtonen and Väre (1998) indicated that the root exudates maintained microbial activities in the nutrient poor lichen-dominated *Picea abies* forest soils in northeastern Fennoscandia. This resembles to our results in the clear-cut area at Mämmilampi. The net decrease of the annual carbon dioxide evolution in the eluvial and illuvial layers was equal to 25% (160 g C m^{-2} $year^{-1}$) in the soil column in the clear-cut area at Mämmilampi. This 25 % contributed to 70% of that in the eluvial and illuvial layers before the clear-cut at Mämmilampi. Behera et al. (1990) showed that the roots contributed to 50% of respiration in forest soil in India. This resembles to our results (25 – 70%) for the Mämmilampi forest soil. The results in Table 3, Paper III, show that the dependence of Mämmilampi soil temperature on air temperature increased after the clear-cutting showing more extreme changes of soil temperature.

The amount of logging residues at Mämmilampi was (6500 g m^{-2}) about 30 fold compared to the annual litter fall in the forest before harvesting. The increase of carbon dioxide evolution in the humus layer was equal to 27% in the soil column in the first year after clear-cutting compared to the year before (Table 12) and may result from an increased substrate feed of remaining logging residues (6500 g m^{-2}) and a change in microclimate (Table 3 in Paper III). Smolander et al. (1998) observed a slight increase of carbon mineralization (25 – 55%) and of microbial biomass carbon and nitrogen in the humus layer in the first summer after clear-cutting in repeatedly N-fertilized, limed, both for those areas treated and those not treated a *Picea abies* soil compared to an uncut area in the same stand. There was no change in the subsequent years (Smolander et al., 1998). Our results (Table 12) and the results of Smolander et al. (1998) showed similar increase of carbon dioxide production in humus layer during the first year after clear-cutting. This was probably due to the increase of substrate from the remaining logging residues, dead roots and change in microclimate, although the amount of root exudates and mycorrhiza may be decreased in the humus layer. Mallik and Hu (1997) reported that endogenous carbon dioxide evolution was similar in the clear-cut and uncut areas in a boreal mixed wood forest in Canada two years after the clear-cutting. This is similar to our endogenous carbon dioxide evolution results (Table 12) from the whole soil column in the clear-cut (Mämmilampi) area during the year before and after the clear-cut. The cumulative activity of sulphatase in Mämmilampi soil column increased by 30% from 8.7 to 11.4 mol m^{-2} $year^{-1}$ after the year of the clear-cut compared to the year before (Table 3 in Paper V, Table 12). Staddon et al. (1998) studied forest soil four years after clear-cutting and found that phosphatase activities were 40% lower, but sulphatase activities did not differ, in the humus layer compared to uncut stand in the same area. Our results (Table 12) and those of Staddon et al. (1998) indicate that clear-cutting affected enzyme activities related sulphur and phosphorus cycling in the soil.

Table 12. Changes in soil activities after harvesting of the *Picea abies* stand at Mämmilampi. The forest was clear-cut in March 1998. The table shows those activities, extrapolated to *in situ* soil temperature, where layer changes were observed before (1997/1998) and after (1998/1999) clear-cutting, of which changed in different directions in the clear-cut area and in the uncut *Pinus sylvestris* stand. The results from the clear-cut area bolded. The activities were calculated to *in situ* soil temperatures (Figure 2 in Paper III) measured on line in each layer at both sites during the whole year.

Change of annual activity in 1998/ 1999 as compared to that in 1997/ 1998						
	Endogenous CO_2 evolution		Methane oxidation		Sulphatase	
	change of yield		change of potential activity		change of potential activity	
	mol m^{-2} $year^{-1}$		mol m^{-2} $year^{-1}$		mol m^{-2} $year^{-1}$	
Soil layer	Hyytiälä	**Mämmi-lampi**	Hyytiälä	**Mämmi-lampi**	Hyytiälä	**Mämmi-lampi**
Humus	+ 3.8	**+ 15**	+ 0.022	**+ 0.004**	- 10.4	**- 1.0**
Eluvial	- 1.3	**- 8.5**	+ 0.032	**- 0.044**	- 7.6	**- 0.1**
Illuvial	+ 1.2	**- 4.8**	- 0.061	**- 0.069**	+ 1.5	**+ 3.8**
Net increase/decrease In the whole soil column	-1.3	**- 1.8**	- 0.007	**- 0.109**	- 17	**+ 2.7**
Total activity of the soil column in 1998/1999	37	**54**	0.322	**0.153**	21	**11.4**
Based on data from	Table 2 in Paper III		Table 2 in Paper III		Table 3 in Paper V	

In the year after the clear-cutting the annual methane oxidation potential calculated to *in situ* temperature decreased from 0.26 to 0.15 mol m^{-2} $year^{-1}$ in the Mämmilampi soil column (17 cm) (Table 2 in Paper III). This decrease was located in the eluvial and illuvial layers (Table 12). Bradford et al. (2000) found that methane oxidation was lowered by 40% - 60% in a clear-cut area of beech and larch forest in the United Kingdom compared to an uncut or a thinned area. This is discussed in more detail in Paper III. These data indicate the importance of tree stands for maintaining methane oxidation potential in forest soil.

When the two years, 1997 / 1998 and 1998 / 1999 are compared (Table 12), it is seen that the annual activities of carbon dioxide evolution, methane oxidation potential and sulphatase changed in different directions at Hyytiälä and at Mämmilampi. The differences may be related to the clear-cutting of the forest at Mämmilampi. These were not explainable by weather conditions between the year 1997 / 8 and 1998 / 9 because the distance between the Mämmilampi and Hyytiälä sites is only 2 km.

5. CONCLUSIONS

1. The chlorine content of sediment organic matter was constant from the depth of 0 cm to 12 cm in the forest Lake Pyylampi, which receives no industrial discharge. A significant part (over 40 %) of the organic chlorine compounds in Lake Pyylampi sediment may have originated from directly or indirectly from the atmospheric fallout as run off from the catchment area of the lake.

2. Organic chlorine content in pulp mill recipient sediment at Vatavalkama in Lake Saimaa had a subsurface maximum. A similar maximum was observed in the industrial recipient in the River Spittelwasser in Bitterfeld. Organic chlorine content in the most polluted sediment layers was 60 – 400 fold higher in the pulp mill recipient area at Vatavalkama and in Spittelwasser than at the same depth in Lake Pyylampi showing heavy and persistent organic halogen pollution in these industrial recipient areas.

3. The highest contents of the twelve inorganic elements analysed from sediments at Vatavalkama were found in layers at 8 – 14 cm depth. This is well below the layers where the highest content of organic chlorine was found, 4 – 6 cm, and shows that the elements were not related to the bleaching of pulp.

4. The linear positive correlation between the activity of butyrate-esterase with sediment ATP content indicates that this activity is suitable for quantifying sediment biomass.

5. In the stratified Lake Pyylampi sediment the activities of β-glucosidase, α-glucosidase, β-xylosidase, sulphatase, aminopeptidase, N-acetyl-glucosamidase and acetate-esterase (mmol m^{-2} cm^{-1} h^{-1}) were remarkably high in the oldest layers (10 – 12 cm). These activities were 20 – 30% of those in the surface sediment (0 – 2 cm). indicating that the organic matter in the oldest sediment layers (10 – 12 cm) was metabolically considerably active.

6. The activity of butyrate-esterase showed no in-depth gradient in the Lake Pyylampi sediment (0 – 12 cm) indicating that all layers (0 – 12 cm) contained a similar density of living biomass.

7. The activities of acetate-esterase, butyrate-esterase, aminopeptidase and sulphatase (mmol m^{-2} cm^{-1} h^{-1}) in the pulp mill recipient sediment at Vatavalkama were lowest in those layers, where the accumulated organically bound chlorine content was highest. These same sediment layers were the most toxic to *Vibrio fischeri* and lowest in ATP indicating severe damage to the microbiological functioning of the sediment.

8. The activity gradients of β-glucosidase, α-glucosidase and β-xylosidase (mmol m^{-2} cm^{-1} h^{-1}) from the surface towards the depth were similar in the pulp mill recipient sediment at Vatavalkama and in the forest Lake Pyylampi indicating that these carbohydratase activities were not sensitive to organic chlorine pollution.

9. Phosphomonoesterase was most active in the heaviest organic chlorine polluted sediment layers (2 – 8 cm) indicating that phosphate was limiting in these layers.

10. The activities of β-glucosidase, β-cellobiosidase, sulphatase, aminopeptidase, N-acetyl-glucosamidase and phosphomonoesterase (mmol m^{-2} cm^{-1} h^{-1}) in the River Spittelwasser sediment in Bitterfeld were lowest in those (6 – 19 cm) layers, where organically bound chlorine content was highest indicating that biodegradation was suppressed in these layers.

11. The activity of butyrate-esterase in Spittelwasser sediment layers was 1/12 – 1/2 of those at the same depths at Vatavalkama or in Lake Pyylampi sediments indicating that the content of living microbial biomass in River Spittelwasser sediment was low.

12. Endogenous carbon dioxide evolution, the activities of β-glucosidase, β-xylosidase, α-glucosidase, β-cellobiosidase, N-acetyl-glucosamidase, aminopeptidase, phosphomonoesterase, acetate- and butyrate-esterase were higher in the humus layer than deeper in soil in the coniferous forest soils in all seasons.

13. Carbon dioxide production per soil organic carbon in the illuvial layer was equal to that in the humus layer of the *Pinus sylvestris* and *Picea abies* forest soils. This indicates similar bioavailability of the soil carbon in surface and subsurface soil.

14. The activities of sulphatase and of aminopeptidase were mainly located below the humus layer in the studied coniferous forest soils. This may indicate that the soil pH was more favourable to these enzymes in the eluvial and illuvial layers than in the humus layer.

15. Methane oxidation located mainly in the illuvial layer in the coniferous forest soils in all seasons. The high activity of the illuvial layer may be related to the higher content of soluble iron, the absence of inhibiting concentrations of ammonium, nitrate or humic substances as well as the more favourable soil pH in illuvial layers than the layers above.

16. N-acetyl-glucosamidase, α-glucosidase, β-xylosidase, sulphatase, aminopeptidase and butyrate-esterase activities per g of soil carbon were the same irrespective of soil depth in *Pinus sylvestris* forest soil. α-glucosidase, β-xylosidase and β-cellobiosidase per g of soil carbon displayed no in-depth gradient in the *Picea abies* forest soil at Mämmilampi. This indicates that the in depth activity gradient observed for several hydrolytic enzymes in forest soils in fact reflected the in depth gradients of organic matter in the same soils.

17. The activities of β-glucosidase, α-glucosidase, β-xylosidase, sulphatase, β-cellobiosidase, phosphomonoesterase, aminopeptidase, N-acetyl-glucosamidase, acetate-esterase, butyrate-esterase and the evolution of carbon dioxide (μmol m^{-2} h^{-1}) were higher or equal in October than in July-August. This indicates that biodegradative activities in soil were regulated by factors other than only soil temperature.

18. The Q_{10}-value of carbon dioxide evolution in coniferous forest soil was 2.3 to 2.8, for methane consumption 2.1 and for β-glucosidase, acetate- and butyrate-esterases between 1.6 and 2.1. This shows that the temperature dependence of these activities was only moderate.

19. During the cold part of the year, from November to April, the coniferous forest soil exhibited 19 – 23% of the annual carbon dioxide evolution, 10 – 14% of the annual methane oxidation potential and 7 – 32% of annual activities of ten hydrolytic enzyme activities, related to carbon, nitrogen, phosphorus and sulphur. These activities, calculated to *in situ* soil temperature, show that coniferous forest soil continued to be metabolically active throughout the winter.

20. The potential for methane oxidation in *Pinus sylvestris* and *Picea abies* forest soils corresponded to less than 1% of the annually endogenously evolved carbon dioxide at *in situ* soil temperature. Methane oxidation potential was more cold sensitive than carbon dioxide evolution.

21. The coniferous forest soils contained an excess of hydrolytic exoenzyme activities compared to the above ground litter fall or to the endogenous carbon dioxide evolution. This indicates that the exoenzyme activities did not limit litter degradation.

22. Methane oxidation potential of *Picea* abies forest soil was suppressed by 40% during the year after the clear-cut of the tree stand, but sulphatase activity benefited from the clearcut. The other nine measured hydrolytic enzyme activities, related to the cycling of carbon, phosphorus and nitrogen, did not respond to clear-cutting within one year. Carbon dioxide evolution activity translocated from the eluvial and illuvial layers to the humus layer during the year after the clear-cutting, possibly in response to the presence of logging residues, decrease in root exudates and changes in microclimate.

6. TIIVISTELMÄ

Tämän tutkimuksen tavoite oli tutkia biohajoavuusaktiivisuuksia eri ekosysteemeissä.Vertailemalla ja yhdistämällä biohajoavuusaktiivisuustietoja saatiin kuva vesistösedimentin ja metsämaan biotoiminnan laadusta, määrästä, toimintaedellytyksistä ja itsepuhdistuskyvystä. Bioaktiivisuudet ovat tärkeitä kasvihuonekaasuihin kuuluvien hiidioksidi- ja metaanilähteiden ja niiden nielujen toiminnassa. Sedimenttien tutkimusalueet sijaitsivat sellutehtaan purkualueella suurjärvessä, Vatavalkamassa ja Tattarissa Saimaalla, saman alueen kahdessa pienessä metsäjärvessä Ruokolahdella ja Saksan itäosassa Spittelwasser-joella teollisuuden vanhalla purkualueella. Kahtatoista bioaktiivisuutta tutkittiin kahdessa havumetsässä Hämeessä, Helsingin yliopiston Hyytiälän metsäasemalla Juupajoella, kaksi vuotta 10 erillisenä mittausjaksona. Podsoloituneen maan eri kerrosten osuus bioaktiivisuuksista, lämpötilavaste, vuodenaikaisvaihtelu ja hakkuuvaste mitattiin. Tutkitut bioaktiivisuudet olivat kymmenen hydrolyyttistä entsyymiaktiivisuutta, jotka liittyvät hiilen, typen, rikin ja fosforin kiertoon, maan hiilidioksidituotanto ja metaaninhapetuspotentiaali.Tulokset ja johtopäätökset ovat seuraavat:

Kerrostuneissa sedimenteissä orgaanisen kloorin pitoisuudet olivat saastuneimmissa kerroksissa sekä sellutehtaan purkualueella (2-6 cm) että teollisuuden purkualueella (6-14 cm) Spittelwasserissa 60-400 kertaa suuremmat kuin kuin samalla syvyydellä metsäjärvessä. Aminopeptidaasin, sulfataasin, asetaatti- ja butyraattiesteraasin aktiivisuudet olivat alimmat orgaanisella kloorilla saastuneimmissa sedimenttikerroksissa (2–6 cm) sellutehtaan purkualueella. Samat sedimenttikerrokset olivat myrkyllisimmät *Vibrio fischeri* testillä mitattuna ja niistä löytyivät alimmat ATP pitoisuudet. Tulokset osoittivat vauriota sellutehtaan purkualueen sedimentin toiminassa. Hiilihydrataasit eivät olleet herkkiä valkaisupäästöille sellutehtaan purkualueella. Sellunvalkaisulla ei tulosten mukaan ollut yhteyttä sedimentin raskasmetallipitoisuuksiin. Sedimenttikerrosten butyraatti-esteraasiaktiivisuudet korreloivat samojen kerrosten ATP pitoisuuksiin indikoiden butyraatti-esteraasin soveltuvan elävän mikrobibiomassan mittariksi. Jokisedimentin β-glukosidaasi, β-sellobiosidaasin, sulfataasin, aminopeptidaasin, N-asetyyli-glukosamidaasin ja fosfomonoesteraasin aktiivisuudet olivat alimmat orgaanisella kloorilla saastuneimmissa kerroksissa indikoiden vauriota sedimentin toiminnassa.

Havumetsämaan hiilidioksidituotanto ja kahdeksan entsyymiaktiivisuutta olivat korkeimillaan humuskerroksessa kaikkina vuodenaikoina. Metaaninhapetuspotentiaalia oli eniten illuviaalikerroksessa. Sulfataasi- ja aminopeptidaasiaktiivisuuksia oli eniten humuskerroksen alapuolella. Hiilidioksidituotanto orgaanisen hiilen pitoisuutta kohden oli yhtäläinen illuviaali- ja humuskerroksessa indikoiden samanlaista hiilen biosaatavuutta. Entsyymiaktiivisuudet ja hiilidioksidituotanto olivat yhtäläisiä tai korkeampia lokakuussa kuin heinä-elokuussa indikoiden niitä kontroloivan myös muut tekijät kuin lämpötila. Havumetsissä kymmenen entsyymiaktiivisuuden vuosisummasta oli 7-32 % kylmänä vuosipuoliskona, marras-huhtikuussa, laskettuna *in situ* lämpötilaan. Vastaavat luvut olivat hiilidioksidituotannolle 19–23 % ja

metaaninhapetuspotentiaalille 10–14 %. Hiilidioksidi ja metaani kuuluvat kasvihuonekaasuihin. Tulokset osoittivat metsämaan olleen metabolisesti aktiivinen kylmänä vuosipuoliskona. Hydrolyyttistä eksoentsyymiaktiivisuuskapasiteettia oli runsaasti enemmän kuin tarvitaan vuotuisen maanpäällisen karikken hajoamiseen ja hiilidioksidin tuotantoon havumetsissä. Tulokset indikoivat, että eksoentsyymiaktiivisuudet eivät olleet nopeutta rajoittava vaihe karikkeen hajoamisessa. Hakkuu alensi metsämaan metaaninhapetuspotentiaalia 40 % vuodessa. Hakkuu nosti sulfataasiaktiivisuutta. Hiilidioksidituotannon vuosisumma pysyi samana hakkuun jälkeen, vaikka se väheni eluviaali- ja illuviaalikerroksessa ja lisääntyi humuskerrokseen liittyen mahdollisesti hakkuutähteisiin.

Metsämaasta ja vesistösedimentistä mitatatut bioaktiivisuudet olivat hyviä metsämaan ja sedimentin toimintaa kuvaavia mittareita. Ne soveltuivat sedimentin tai maaperän metabolisen koneiston vaurioiden toteamiseen ja palautumisen seurantaan.

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