Department of Environmental Sciences
Faculty of Biological and Environmental Sciences
University of Helsinki, Lahti
Finland

MICROBIAL COMMUNITY COMPOSITION IN VARIOUS SOILS AND GROUNDWATER DEPOSITS AND EFFECTS OF LONG-TERM PESTICIDE CONTAMINATION

Marja Mattsson

ACADEMIC DISSERTATION IN ENVIRONMENTAL ECOLOGY

To be presented, with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki, for public criticism in the Auditorium of Lahti Science and Business Park, Niemenkatu 73, Lahti, on January 22nd at 12 o'clock noon.

Lahti 2016

Supervisors Docent Merja Kontro
Department of Environmental Sciences
University of Helsinki
Lahti, Finland
Professor Martin Romantschuk
Department of Environmental Sciences
University of Helsinki
Lahti, Finland

Reviewers Professor Erland Bååth
Department of Biology
Lund University
Lund, Sweden

Professor Olli H. Tuovinen
Department of Microbiology
Ohio State University
Columbus, OH, USA

Opponent Docent Hannu Fritze
Natural Resources Institute Finland
Jokioinen, Finland

Custos Professor Rauni Strömmer
Department of Environmental Sciences
University of Helsinki
Lahti, Finland

ISBN 978-951-51-1837-0 (paperpack)
ISBN 978-951-51-1838-7 (PDF; http://ethesis.helsinki.fi)
ISSN 1799-0580

Unigrafia
Helsinki 2016

CONTENTS

LIST OF ORIGINAL ARTICLES

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I. Talja* KM, Kaukonen S, Kilpi-Koski J, Malin I, Kairesalo T, Romantschuk M, Tuominen J, Kontro MH. 2008. Atrazine and terbutryn degradation in deposits from groundwater environment within the boreal region in Lahti, Finland. J Agric Food Chem 56: 11962-11968, DOI: 10.1021/jf802528a

II. Mattsson, KM, Liu X, Yu D, Kontro MH. 2015. Depth, soil type, water table, and site effects on microbial community composition in sediments of pesticide-contaminated aquifer. Environ Sci Pollut Res 22: 10263-10279, DOI: 10.1007/s11356-015-4224-1

III. Mattsson KM, Kontro MH. Microbial community composition in groundwater monitoring pipe deposits with and without atrazine contamination, according to lipid biomarkers. Manuscript

IV. Mattsson KM, Närhi I, Kontro MH. Microbial community structure in pesticide-contaminated market garden soils. Manuscript

Previously published papers were reproduced with the kind permission of American Chemical Society (I) and Springer (II).

*) Mattsson, previously Talja

AUTHOR´S CONTRIBUTION

I. Corresponding author. IM chose sampling sites, SK set up laboratory experiments, JKK collected the samples, TK, MR, and JT helped in practical arrangements. MKM (MKT) participated in guiding the laboratory analyses, calculated the results, analyzed the data, and wrote the article with MHK.

II. Corresponding author. XL collected the samples, DY analyzed pesticides. MKM took care of lipid analyses, calculated the results, analyzed data, and wrote the article together with MHK.

III. Corresponding author. MKM was responsible of taking care lipid analyses, calculated the results, analyzed the data, and participated in writing the article together with MHK.

IV. Corresponding author. IN collected the samples. MKM performed a part of chemical analyses, guided lipid analyses, calculated the results, analyzed the data, and wrote the article with MHK.

ABSTRACT

The microbial community composition is among the most important factors to consider, when pesticide removal from the environment is planned by indigenous microbial consortia. The main interest of this study was to assess relationships between pesticide contaminations from the past, microbial community compositions, physico-chemical conditions, and pesticide dissipation. The microbial communities were studied by lipid biomarkers. Samples were collected from surface soils to deep drilling sediments and groundwater deposits, which had the long history of pesticides such as atrazine, 2,6-dichlorobenzamide, simazine, 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT), hexachlorobenzene (HCB), and pentachloroaniline (PeCA). The microbial lipid quantities and community compositions were generally dependent on organic matter (OM) content and soil type. Microbial biomass and cell numbers decreased with increasing depth and varied more on the top soil layers than in the deep sediments. Generally, the quantity of phospholipid fatty acids (PLFAs) was higher than that of glycolipid fatty acids (GLFAs) but lower than the quantity of neutral lipid fatty acids (NLFAs), when the amounts of main nutrients were balanced. However, in groundwater deposits and deep drilling sediments the phosphorous deficiency possibly caused higher GLFA quantities compared to PLFAs. The quantity of NLFAs seemed to increase with soil OM content and excess carbon.

Microbes in all polluted samples seemed to be adapted to pesticides. Pesticide impacts on microbial community composition were few and no stress were found in the physiological indices of lipid profiles. The long-term contamination of atrazine in the groundwater, as well as DDT, HCB, and PeCA in surface soils seemed to mainly increase microbial activity compared to controls. The effects of DDT and its residues on microbial lipids were minor, though their persistence was high in soils with low OM content. HCB and PeCA were more persistent in soils with high OM content and their effects on microbial lipids were more numerous. The indigenous microbes of groundwater pipe A deposits were capable of atrazine biodegradation in aerobic conditions though nutrient levels were low, when C/N-ratio was near to optimal. Possibly Gram-positive bacteria and fungi were related to atrazine biodegradation.

TIIVISTELMÄ

Kun suunnitellaan torjunta-aineiden poistamista maaperästä ja pohjavedestä mikrobien avulla, on kohteen alkuperäisen mikrobiyhteisön rakenteen tunteminen tärkeää. Tämän tutkimuksen päätavoitteena oli selvittää vuorovaikutuksia kauan jatkuneen torjunta-aineiden aiheuttaman pilaantumisen, mikrobiyhteisön rakenteen, maaperän ja pohjavesiympäristön koostumuksen ja torjunta-aineiden hajoamisen välillä. Mikrobiyhteisön rakennetta tutkittiin lipidibiomarkkerien avulla. Tutkitut näytteet sisälsivät nykyisin EU-maissa kiellettyjä torjunta-aineita kuten atratsiini, simatsiini, 2,6-diklooribentsamidi, 1,1,1-trikloori-2,2-di(4-kloorifenyyli) etaani (DDT) ja sen hajoamistuotteet 1,1-dikloori-2,2-di(4-kloorifenyyli)etaani (DDD) ja 1,1-dikloori-2,2-di(4-kloorifenyyli)etyleeni (DDE), heksaklooribentseeni (HCB) ja pentakloorianiliini (PeCA, pentakloori-nitrobentseenin hajoamistuote).

Maan orgaanisen aineen määrällä ja maalajilla oli suurin vaikutus mikrobien biomassaan ja mikrobiyhteisön rakenteeseen. Mikrobien biomassa ja solujen lukumäärä vähenivät syvyyden kasvaessa, vaikka mikrobimäärien vaihtelu oli syvällä vähäisempää kuin pintamaassa. Torjunta-aineiden vaikutus oli havaittavissa lähinnä mikrobipopulaatioiden adaptaationa torjunta-aineille. Lisäksi mikrobien aktiivisuus oli korkeampi pilaantuneissa maissa kuin puhtaissa.

DDT ja sen hajoamistuotteet DDD ja DDE säilyivät vuosikymmeniä vanhojen taimitarhojen pintamassa. Hiekkamaassa, jossa orgaanisen aineen määrä oli pieni, DDT-pitoisuudet olivat suurempia ja vaikutukset mikrobiyhteisön rakenteeseen vähäisemmät kuin enemmän orgaanista ainetta sisältävässä maassa. PeCA ja HCB osoittautuivat yhtä pitkään säilyviksi kuin DDT, mutta toisin kuin DDT, niiden pitoisuudet olivat suurempia ja vaikutukset mikrobiyhteisöön lukuisammat niissä maissa, joissa oli enemmän orgaanista ainetta.

Pohjaveden omat mikrobit, jotka oli otettu yhdestä pohjaveden näytteenottoputkesta, pystyivät laboratoriokokeissa hajottamaan atratsiinin nopeasti, kun muissa pohjavesiputkissa atratsiinin hajoaminen oli hidasta. Atratsiinin hajoaminen tapahtui hapen läsnä ollessa, vaikka ravinnetaso oli alhainen, mutta hiili-typpi suhde oli lähellä optimaalista. Gram-positiivisten bakteerien ja sienten määrät olivat korkeammat atratsiinia hajottaneessa mikrobiyhteisössä kuin muissa pohjavesiputkissa.

ABBREVIATIONS

BAM	2,6-dichlorobenzamide
cy/pre	the ratio of cyclopropyl fatty acids to their monoenoic precursors
DDD	1,1-dichloro-2,2-di(4-dichlorophenyl)ethane
DDE	1,1-dichloro-2,2-di(4-dichlorophenyl)ethylene
DDT	2,4-DDT:1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane
	4,4-DDT: 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane
DEA	desethylatrazine
DEDIA	desethyldeisopropylatrazine
DIA	deisopropylatrazine
EU	European Union
FA	fatty acid
GL	glycolipid
GLFA	glycolipid fatty acid
HCB	hexachlorobenzene
K-W	Kruskal-Wallis test
MW	molecular weight
M-W	Mann-Whitney test
NL	neutral lipid
NLFA	neutral lipid fatty acid
OM	organic matter
PCA	principal component analysis
PCNB	pentachloronitrobenzene
PeCA	pentachloroaniline
PL	phospholipid
PLFA	phospholipid fatty acid
s.d.	standard deviation
SRB	sulfate reducing bacteria
TBSA	tuberculostearic acid
unsat/sat	the ratio of unsaturated fatty acids to saturated fatty acids

1 INTRODUCTION

1.1 PESTICIDES IN THE ENVIRONMENT

Pesticides are used for weed, insect, and fungal control largely in agricultural areas and in gardens, railways, roads, and industry (Okutman Tas and Pavlostathis 2007). Pesticide use is troublesome due to toxicity, persistence, and the rise of resistant species in ecosystems. Though the properties of pesticides, such as chemical structure, functional groups, molecular weight, solubility, vapor pressure, toxicity, and sorption to particles, determine the major part of the pesticide outcome in the environment, soil or sediment characteristics, such as pH, humus content, clay, and iron oxides influence the pesticide behavior in ecosystems (Aislabie and Lloyd-Jones 1995, Welp and Brümmer 1999, Johnsen et al. 2001, Qui and Davis 2004). There is a consensus among researchers that microbial degradation is the principal removal process for most pesticides in the environment (Aislabie and Lloyd-Jones 1995, de Lipthay et al. 2004, Holden and Fierer 2005). This biodegradation process involves the break-down of chemical bonds of the compound to smaller units by microbial enzymes (Atlas and Bartha 1998). Besides microbes carrying appropriate enzymes, a number of environmental factors must be favorable to biodegradation; e.g. soil type, moisture, pH, temperature, salinity, redox-potential, nutrient availability, alternative carbon sources, and electron acceptors have their influences on the biodegradation rate. In addition, microbes or microbial enzymes must be in contact with the pesticide (Aislabie and Lloyd-Jones 1995, Briceño et al. 2007). Biodegradable pesticides may serve as an available source of nutrients, like carbon and nitrogen, to microorganisms, and stimulate bacterial productivity (Haney et al. 2000, Downing et al. 2004, Shapir et al. 2007).

The degradation products of pesticides, as well as the parent compounds, may be found in air, soils, sediments, surface and ground waters, and in non-target organisms even decades after pesticide application in the environment (Meijer et al. 2001, Jablonowski et al. 2008). This concerns especially recalcitrant chlorinated compounds, such as insecticide 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT), fungicides hexachlorobenzene (HCB), and quintozene, which is the trade name for pentachloronitrobenzene (PCNB), and degradation products (Stevens et al. 2003, Katsoyiannis and Samara 2004, Gaw et al. 2005, Muir and Sverko 2006, Okutman Tas and Pavlostathis 2007, Eganhouse and Pontolillo 2008). A herbicide atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine has been one of the most common control agents throughout the world for over 50 years. Though the sale of atrazine has been forbidden in European Union (EU) countries due to its carcinogenicity, it remains in use in many major agricultural countries, including

USA and China (Johnsen et al. 2001, Huang et al. 2003, Downing et al. 2004, Krutz et al. 2010). The slowly degradable s-triazines may leach to groundwater, and may be a threat for human health in drinking water resources.

1.2 NATURAL MICROBIAL COMMUNITIES

1.2.1 Microbial communities in surface and subsoil

Soil represents diversity higher than any other natural habitat supporting the growth of 10^7–10^{10} prokaryotic cells per gram dry soil and biomass of 300 to 3000 kg per ha (Ranjard and Richaume 2001, van Elsas et al. 2007). Soil is an extremely heterogeneous, structured and mainly nutrient-poor habitat. The chemical, physical, and biological characteristics of soil are changing in time and space (Nannipieri et al. 2003). Soil organic matter or carbon is considered to be the major factor regulating the size of the microbial biomass (Sylvia et al. 2005, Fierer et al. 2009). However, soil type, season, land management, soil pore and aggregate size, water availability and disturbance, and plant species and diversity are also affecting variations in microbial biomass (Zelles et al. 1995, Bossio et al. 1998, Grayston et al. 2001, Habekost et al. 2008, Drenovsky et al. 2010). The spore forming bacteria, especially *Streptomycetes* and *Bacillus* spp., can be transported with water from surface soil to subsurface layers (Balkwill et al. 1997). The microbial biomass and cell numbers are shown to decrease with depth, but increase with layer age (Balkwill et al. 1997, Taylor et al. 2002, Allison et al. 2007). Microbial biomass is generally 2–6 orders of magnitude lower in subsurface than in top soils, comprising 10^3 to 10^6 pmol per gram (Brockman and Murray 1997, Pfiffner et al. 2006). Biomass quantity has been shown to drop by an order of magnitude at the depth 0.8 m, which is a typical phenomenon for microbial distribution in soils (Federle et al.1986).

Microbial diversity or biodiversity describes the complexity and variability at all levels of biological organizations, including genetic distribution within species or populations, the abundance of species in communities, variations in community composition, and abundance of guilds and trophic levels in ecosystems (Torsvik and Øvreås 2002, Nannipieri et al. 2003). An important aspect of diversity at the ecosystem level is the functional diversity, which can be expressed as the number of different functional groups fulfilling different physiological and metabolic functions in the community (Torsvik and Øvreås 2007). Many investigators have reported that ecological functions of different bacteria may overlap in the community (Botton et al. 2006). Within prokaryotic populations with genetic variations created by mutations and genetic recombination, natural selection causes high biodiversity in complex soil ecosystems (Torsvik and Øvreås 2007, Griebler and Lueders 2009). Due to the huge microbial diversity in soil, it might be more practical to determine how the

microbial diversity changes across the heterogeneity of different soil habitats instead of the actual diversity of microbial communities (Hughes et al. 2001).

Microbial community structure can be defined as the number and relative abundance or biomass of microbial populations, such as fungi, and Gram-negative and Gram-positive bacteria (Jörgensen and Emmerling 2006). At site level, abiotic and biotic heterogeneity has been identified as an important driver of soil microbial communities, which may be similar in different regions with similar environmental gradients (Johnsen et al. 2001, Fierer et al. 2009). The bacterial community composition can be explained mainly by soil pH (Bååth and Anderson 2003, Fierer and Jackson 2006, Lauber et al. 2009), depth (Griffiths et al. 2003), soil texture (Brockman and Murray 1997, Johnsen et al. 2001, Lauber et al. 2008), and in the boreal region also by C/N ratio (Högberg et al. 2007).

Generally, Gram-negative bacteria are considered to be opportunistic, fast-growing, and requiring access to readily available organic matter (r-strategists), while Gram-positive bacteria are mostly oligotrophic, slow-growing, and surviving with a low supply of nutrients (K-strategist), although there are exceptions (van Elsas et al. 2007). Gram-negative bacteria generally live in surface soils, in the rhizophere using plant biomass as a carbon source, while Gram-positive bacteria are also found in deeper soil layers using older soil organic matter or recalcitrant compounds (Fierer et al. 2003, Kramer and Gleixner 2008), and in saturated wetlands (Bossio et al. 2006). Gram-positive bacteria and actinobacteria have high resistance to fluctuating environmental conditions, whereas Gram-negative bacteria have low resistance to stress factors such as desiccation (Ranjard and Richaume 2001, Blume et al. 2002, Fierer et al. 2003). Though sulfate-reducing bacteria (SRB) have been abundant in anoxic subsurface soils, they are also detected in oxic habitats (Muyzer and Stams 2008).

Gram-negative bacteria or actinobacteria have been detected in natural areas without plants, while more fungi have been found in relation to bacteria in more complex plant communities (Bardgett et al. 1999, Pennanen et al. 2001, Zak et al. 2003, Brant et al. 2006). Soil of coniferous forest has been demonstrated to carry particularly high fungal to bacterial ratios due to occurrence of ectomycorrhizal fungi (Frostegård and Bååth 1996), while the lowest ratios have been found in soils from non-vegetated areas, deserts, and grassland indicating bacterial dominance (Bardgett 2005). In agricultural areas, the microbial biomass and abundance of fungal populations have been lower than in natural areas (Frostegård and Bååth 1996, Allison et al. 2005), and in greenhouses the microbial biomass has been lower than in field soil (Ibekwe and Kennedy 1998). The abundance of fungi decreases with depth more than that of bacteria (Holden and Fierer 2005).

1.2.2 Microbial communities in groundwater environments

Groundwater is defined as "sub-surface water in soils and geologic strata that have all their pore spaces filled with water" (Jolly et al. 2008). Pristine groundwater provides few resources for microorganisms and their biomass is low, such that the cell number may vary between 10^2–10^6 cells ml^{-1}, and 10^4–10^8 cells cm^{-3} of sediment (Balkwill et al. 1988, Green and Scow 2000, Goldscheider et al. 2006, Pedersen et al. 2008, Griebler and Lueders 2009). The majority of aquifer microbes are attached to sediment or aquifer materials, and the attached and detached cells have different lipid profiles (Tunlid et al.1989, Green and Scow 2000, Goldscheider et al. 2006). In aquifers, microbes are generally viable, but not cultivable (Green and Scow 2000).

In groundwater environments, microbial communities mainly consist of organoheterotrophic microbes adapted to this stable oligotrophic environment, and also lithoautotrophic bacteria oxidizing inorganic compounds, such as manganese and sulfur. In addition, iron- and sulfate reducing, nitrifying, fermentative, methanogenic, and acetogenic organisms are detected (Krumholz 2000, Pedersen 2008, Griebler and Lueders 2009). Most bacteria have been Gram-positive, while Gram-negative bacteria display nutrient stress (Balkwill et al. 1997). Surface water is filtrated through the upper layers of soil, and transports microbes and substrates to deeper deposits in the formation of groundwater. The indigenous groundwater microbial community compositions differ from those in surface soils, varying in phylogenetic composition and physiological capacities (Griebler and Lueders 2009). Though the biodiversity in isolates from aquifers is low, spatial heterogeneity between sediment minerals, the availability of oxidable compounds and organic matter, local hydrogeochemical properties, and sediment grain sizes may increase microbial diversity in aquifers (Zhou et al. 2002, Griebler and Lueders 2009). The temperature adaptation in bacterial cells can be seen in changes in the degree of unsaturation and branching, cyclization, and chain lengths of fatty acids in cell membranes (Suutari and Laakso 1994).

1.3 EFFECTS OF DISTURBANCES ON MICROBIAL COMMUNITIES

Environmental disturbances can be natural, such as flood, fire, and drought, or anthropogenic, like toxic substance discharge to the environment. They can cause decreased microbial production and biodiversity changes in microbial community composition, which can be seen even decades after the anthropogenic disturbance (Odum 1985, Atlas et al. 1991, Rapport and Whitford 1999, Buckley and Schmidt 2003, Haack et al. 2004). When toxic stress occurs in an ecosystem, the functions are maintained by the replacement of sensitive with tolerant populations. Researchers have reported shifts in microbial community composition from Gram-positive bacteria and fungi to Gram-negative bacteria and actinobacteria (Kalia and Gosal

2011, Jacobsen and Hjelmsø 2014), or increased abundance of Gram-positive bacteria with metal pollution in soil (Pennanen et al. 1996). However, the disturbed populations have shown a large range of substrate utilization, metabolic versatilities, and increased physiological tolerance (Atlas et al. 1991).

Responses of microbial populations to disturbances may include resistance or resilience (Griffiths et al. 2001, Westergaard et al. 2001). When the microbial community is not altered after a disturbance, it is considered to be resistant. When the community changes, but recovers and returns to the original state, it is resilient. However, microbial communities may change in composition without any effects on microbial processes, different microbial groups carrying out the same functions, which is called functional redundancy (Allison and Martiny 2008).

Though diverse communities have been considered to be more resistant to environmental disturbances (Peterson et al. 1998), the high diversity does not necessarily ensure functional stability in microbial communities. It has been shown that, in most cases, perturbations can alter microbial community structure and this might change microbial processes like nutrient cycling, decomposition, and energy flow. The communities have not reverted to the composition prior to the disturbance, though the intensity of effects and the rate of recovery have been reported to be dose-dependent (Allison and Martiny 2008, Ager et al. 2010). The functional processes of the community are crucial to maintain, not the species composition (Øvreås 2000). However, any shifts in community structure will have consequences on ecosystem function, when the tolerant microorganisms fail to compensate for biogeochemical functions normally carried out by inhibited or eliminated microbial groups (Widenfalk et al. 2008).

The diversity index of Shannon-Weaver (H´) has been used to describe the effects of disturbances on microbial diversity, possibly indicating altered ecosystem processes (Hedrick et al. 2000, Humbert and Dorigo 2005). On the other hand, the changes in the microbial community composition rather than diversity have been suggested to estimate the disturbance effects, since these sensitive changes are long-lasting (Westergaard et al. 2001). Shannon-Weaver index calculated by PLFAs has been criticized in the literature, as it does not give a correct perception of the diversity of microbial species (Frostegård et al. 2010).

1.4 THE INFLUENCE OF PESTICIDES ON MICROBIAL COMMUNITIES

Many types of pesticides like cyclodiene, organochlorine, organophosphorous, phenylurea, phtalimid, and triazine pesticides have been found to have little or no effects on microbial biomass; however, the enhancement of resistant microbes has been documented (Downing et al. 2004, Widenfalk et al. 2008). The abundance of

Gram-negative bacteria has increased more than that of Gram-positive bacteria at low concentrations of pesticides, due to their ability to use rapidly different nutrient sources (Das and Mukherjee 2000, Wang et al. 2008). However, high concentrations of chlorobenzene and phenoxycarboxylic acid have been more toxic to Gram-negative bacteria than Gram-positive bacteria (Thompson et al. 1999, Zhang et al. 2010). In sediments, Gram-positive bacteria have increased in consequence of pesticide pollutions (Widenfalk et al. 2008). The influence of pesticides on fungal populations has been slight or fungal growth inhibiting (Ratcliff et al. 2006, Wang et al. 2008, Widenfalk et al. 2008).

In laboratory studies, the application of herbicide atrazine has increased atrazine-degrading microbial populations and enhanced degradation (Rhine et al. 2003, Ros et al. 2006, Krutz et al. 2010). In field experiments, the soil type had an effect greater than herbicide, e.g. atrazine on the microbial community structure (Seghers et al. 2005). The long-term use of atrazine has altered the bacterial community structure of methanotrophs without changes in bacterial activity (Seghers et al. 2003). High concentrations of organic matter together with atrazine contamination may increase populations of atrazine degraders or overall microbial populations (Holden and Fierer 2005).

DDT application has reduced bacterial populations, and increased fungal populations, while DDT-resistant microbes have transformed DDT to DDE or DDD (Megharaj et al. 2000, Kantachote et al. 2001, 2003). Exposure to 1,2-dichlorobenzene has decreased soil fungal populations (Thompson et al. 1999).

In groundwater environments, pesticide contaminations can increase microbial activity, cell numbers, and biomass, and change microbial community structure (Pfiffner et al. 1997, de Lipthay et al. 2004, Humphries et al. 2005, Goldscheider et al. 2006). The bacterial diversity (H´) under pesticide exposure has increased, decreased, or remained unaffected (Pfiffner et al. 1997, de Lipthay et al. 2004, Johnson et al. 2004, Humphries et al. 2005). However in groundwater environments, an increase in the microbial abundance in consequence of a substrate input, like pesticides, is more likely (Griebler and Lueders 2009).

The effects of pesticides on soil microbial community structure are the predominant interest when pesticides are removed by bioremediation. Microbial evolution with pesticide degraders can be expected with contamination of pesticides and other xenobiotics (Pombo et al. 2005, Weiss and Cozzarelli 2008). Researchers have documented the degradation genes evolving, and horizontal gene transfers within bacteria degrading chlorinated compounds in aquifers or atrazine in soils (Van der Meer et al. 1998, Krutz et al. 2010). The evolution in groundwater environments may be slower than in the upper soil layers, due to small population sizes, slight microbial activity, and stable conditions (Griebler and Lueders 2009). However, it is likely that natural biodegradation of pesticides by indigenous microbes also occurs in aquifers (Weiss and Cozzarelli 2008). Besides indigenous microbes, the soil

conditions must be satisfactory for biodegradation, including nutrient availability, temperature, water content, amount of oxygen, and soil pH (Briceño et al. 2007).

1.5 LIPID PROFILES IN MICROBIAL COMMUNITY STRUCTURE INVESTIGATIONS

Lipids comprise less than 5 % of bacterial dry weight (Lechevalier 1977). The analysis of lipids as signature biomarkers has frequently included di- and triacylglycerols, phospholipid fatty acids (PLFAs), neutral lipid fatty acids (NLFAs), and lipopolysaccharide hydroxy fatty acids (White et al. 1998, Bååth 2003, Keinänen et al. 2003). The FA composition varies significantly from one organism to another, and more than 200 different FAs have been detected from microorganisms. The PLFAs are considered to be components of viable microbial populations, due to the presence in membranes of all living cells, but not in storage lipids or in dead cells. Furthermore, the PLFAs allow the description of whole microbial communities (Zelles 1999). The bacterial cells contain straight-chain saturated and monounsaturated, branched chain, cyclopropyl, and polyunsaturated FAs, mostly 10 to 20 carbons long (Green and Scow 2000).

The technique for the determination of microbial lipids differentiates phospholipid, glycolipid, and neutral lipid FAs from living bacterial and fungal cells in the soil, and provides a microbial community fingerprint for a sample. The FA profiles have proven to be practical for the detailed characterization of changes in mixed microbial communities in the environment, especially for screening indicators of environmental disturbances (Torsvik and Øvreås 2007). Changes in lipid profiles may indicate either changes within the microbial community or changes in the specific microbial group (Frostegård et al. 1993, Zelles 1999).

In addition, physiological and nutritional information from microbial communities can be obtained from PLFAs (White et al. 1998, Zelles 1999, Green and Scow 2000). In the literature, an increase in cyclopropane FAs and the ratio of cyclopropyl FAs to their monoenoic precursors have commonly been indicators of stress and slow growth (Kieft et al. 1994, Bossio et al. 2006). The ratio of *trans*- to *cis*-monoenoic FAs has been related to signs of toxic stress and starvation within Gram-negative bacteria (Kieft et al. 1994, Bossio and Scow 1998, Cronan 2002, Heipieper et al. 2003), but they have proven to be unreliable (Fischer et al. 2010). Bacteria may modify their membrane fluidity as a response to environmental changes, which can also be detected in the regulation of the ratios of saturated to unsaturated PL FAs, branched to unbranched PL, *iso*-branched to *anteiso*-branched FAs, and in changes in the acyl-chain length (Sikkema et al. 1995, White et al. 1996, Šajbidor 1997, Mrozik et al. 2005, Nielsen et al. 2005, Grandvalet et al. 2008). The reduction in membrane fluidity of Gram-negative bacterial cells has been considered

a technique to prevent toxic chemical compounds to penetrate the cells, which allows microbes to survive in contaminated environments (Sikkema et al. 1995, Weber and de Bont 1996, Ramos et al.1997).

The limitation of PLFAs is that many phylogenetic and physiological microbial groups lack specific lipid biomarkers (Weiss and Cozzarelli 2008). Nevertheless, PLFAs have also been used to determine total microbial biomass for samples (White et al. 1979, Zelles 1999, Green and Scow 2000). Microbial biomass pmole PLFA g^{-1} soil has been found to correlate significantly with contaminant concentrations, which could be used in the monitoring of bioremediation (Ringelberg et al. 2008, Weiss and Cozzarelli 2008).

Glycolipid FAs (GLFAs), like PLFAs, depend on the availability and structure of carbon source (Wick et al. 2003). The high amount of GLFAs is typical for Gram-positive bacteria (Kontro et al. 2006). There is a lack of information on changes in GLFAs as a response to alterations in the environment. Neutral lipids, such as triacylglycerols, are storage compounds in eukaryotic organisms (Bååth 2003). Fungi can accumulate triacylglycerols in the absence of phosphorous and nitrogen, and presence of an excess of carbon (Bååth 2003, Kontro et al. 2006). High proportions of neutral lipid FAs (NLFAs) could indicate prominent fungal populations or dead bacterial cells whose PLFAs have become a part of neutral lipids (Ruess and Chamberlain 2010).

2 THE AIMS OF THE STUDY

The hypothesis of this study was that recalcitrant pesticides have long-lasting effects on microbial community composition, while the null hypothesis was that no effects can be elucidated. The main objective of this work was to investigate whether long-term pesticide contamination has caused permanent changes in microbial community composition. The study sites were groundwater-monitoring-pipe and well deposits, sediments from two drillings, and soils from old market gardens, all included pesticide contaminated samples. More specifically, the main issues were:

1. Characterizing microbial community compositions and factors affecting it in the groundwater deposits, drilling sediments, and surface soils, all included long-term contaminated samples with pesticides (atrazine, simazine, BAM, DDT, HCB, and pentachloroaniline) (II, III, IV)

2. Evaluating changes in microbial community compositions due to the pesticide application in the surface soils and subsurface environments (III, IV)

3. Evaluating the dependence of pesticide degradation (bioremediation) on the microbial community compositions, and environmental factors in groundwater deposits, drilling sediments, and soils (I, II, III, IV)

3 MATERIALS AND METHODS

3.1 SAMPLINGS

Groundwater deposits were taken from well W and two groundwater monitoring pipes (B, C) located in the aquifer with atrazine and degradation products desethylatrazine (DEA) and deisopropylatrazine (DIA) in the groundwater of Lahti, southern Finland (Table 1). Water from control pipe D from the same aquifer, as well as water from control pipe A from the adjacent aquifer, was clean. The depths of the sampling sites varied from 6.3 m to 21.0 m below the surface (mbs), and 0.8–16.9 m below the water table (mbt). Drilling sediments were taken from layers that were contaminated with herbicides atrazine, 2,6-dichlorobenzamide, (BAM, a degradation product of dichlobenil), and simazine (Table 1). The depths of the drilling sites varied between 0.3 mbs to 16.7 mbs next to the railway (R1-R4), and between 0.8–24.8 mbs in the city garden (G1-G6). The surface soils from the depths of 0.1–1.3 m were collected in abandoned market gardens from three sampling sites in western Finland. Some samples were contaminated with DDT, hexachlorobenzene (HCB), and/or pentachloroaniline (PeCA, a degradation product of pentachloronitrobenzene, PCNB) (Table 1).

Table 1. Sites and times of sampling (Roman numbers refer to the original articles).

Sampling site	Abbreviation	Sampling time
Groundwater deposits, Lahti (I, III) (monitoring pipes and well)	A, B, C, D, Well	May 2003
Drilling sediments, Lahti (II) (Garden sediments) (Railway sediments)	 G1-G6 R1-R4	 August 2005
Surface soils, (Market garden) (IV) Kokkola Alahärmä Kuortane	 A1-A6 B1-B7 C1-C6	 July 2006 early August 2006 late August 2006

3.2 BACKGROUND INFORMATION ON PESTICIDES

The sale of pesticides has been under control in Finland since 1952. The insecticide DDT came into the market in 1946, and was sold approximately 149 tons in 1953–1972 (Närhi 2008). The sale of DDT was banned in Finland in 1976. Quintozene (PCNB) was approved for use in fungicide in greenhouses in 1963, however its

use was limited since 1975 and the sale was banned in 1991. The use of herbicide simazine began in Finland in 1959, and that of atrazine in 1962. Atrazine was widely used herbicide, which recommended application rate was 3–4 kg per hectare, while that of simazine was 5–6 kg per hectare. Herbicide dichlobenil was used in tree planting areas since 1980s (Jaakkonen and Sorvari 2006). The sale of atrazine was forbidden in 1992, that of simazine in 2004 and dichlobenil in 2011.

3.3 CHEMICAL ANALYSES

Pesticides from groundwater were analyzed with solid phase extraction, followed by high resolution gas-liquid chromatography – mass spectrometry performed by Ramboll Analytics Ltd in Lahti, Finland (I, III), and from market garden soils using a multi residue method performed by Lantmännen AnalyCen laboratory in Tampere, Finland (IV). Pesticides from the liquid samples of degradation experiments and extracts of drilling sediments were analyzed by HPLC in the department of Environmental Sciences in Lahti (I, II).

3.4 LIPID ANALYSES

Lipids from the samples were extracted as originally described by Bligh and Dyer in 1959 (II, III, and IV). Duplicate samples were extracted in chloroform : methanol : phosphate buffer (1 : 2 : 0.8 vol/vol/vol). An internal standard of dipentadecanoylphosphatidylcholine was added for quantification of phospholipids. Lipids were separated from the solvent phase after adjusting the ratio of chloroform : methanol : buffer to 1 : 1 : 0.9 vol/vol/vol. The dried lipid extract was dissolved in chloroform, and applied to a 10 ml Varian column (Varian, Las Vegas, NV, USA). The lipids were fractionated to neutral, glyco- and phospholipids with 10 ml of chloroform, 20 ml of acetone, and 10 ml of methanol, respectively. Internal standards of tridecanoic acid methyl ester and nonadecanoic acid methyl ester were added to phospho-, glyco-, and neutral lipid fractions. FAs were saponified, methylated, and extracted as methyl esters (Kontro et al. 2006). The FA methyl esters were analyzed with a Shimadzu gas chromatograph model GC-17A (Duisburg, Germany) equipped with a mass selective detector (model GCMC-QP5000) and automatic sampler, model AOC-17 (Kontro et al. 2006).

3.5 CALCULATIONS

Lipid biomarkers were used for the classification of soil bacteria and fungi. Saturated straight-chain FAs (14:0, 16:0, 17:0, 18:0, 20:0) were used for general biomass; monounsaturated straight-chain phospholipid FAs (16:1ω9c, 16:1ω7c, 16:1ω7t, 16:1ω5c, 18:1ω9c, 18:1ω7c, 18:1ω7t, and 18:1ω5c) were ordinarily indicators for Gram-negative bacteria in groundwater and drilling sediments; in surface soils, 18:1ω9c and 18:2ω6c were used for fungi; *iso*- and *anteiso*-branched FAs (*i*-14:0, *i*-15:0, *i*-16:0, *a*-15:0, *i*-17:0, *a*-17:0, *i*-18:0) for Gram-positive bacteria; cyclopropane FAs (cy-17:0ω9c, cy-17:0ω7c, cy-19:0ω9c, cy-19:0ω7c) for Gram-negative and lactic acid bacteria; 10Me16:0 for sulfate reducing bacteria (SRB); and 10-methyloctadecanoic acid (tuberclostearic acid, TBSA) for actinobacteria (White et al. 1996, Frostegård and Bååth 1996, Zelles 1999, Bossio et al. 2006). Cyclopropane FAs, the ratios of cyclopropyl FAs to their monoenoic precursors (cy-17:0ω7c, /16:1ω7c, cy-17:0ω9c /16:1ω9c, cy-19:0ω7c/18:1ω7c, and cy-19:0ω9c/18:1ω9c), unsaturated to saturated FAs, and FA chain length evaluated from the molecular weight of FAs were used as indictors for stress and slow growth (Kieft et al. 1994, Bossio and Scow 1998). Physiological indicators were examined in order to clarify the responses of microbial communities for depth, soil type, and pesticide contamination. Average carbon number, cy17:0/pre, cy19:0/pre, unsat/sat, and the ratios of *i*/*a*-15:0 and *i*/*a*-17:0 were used as physiological indicators. Average values and standard deviations of the percentage FAs and indicators were calculated from the sites to be compared. Microbial cell numbers were calculated as presented in II.

3.6 COMPARISONS

To determine the impact of depth on microbial community composition, the uncontaminated sandy surface soils (A1, B5) from the depth of 0.1–0.3 m, were compared to the uncontaminated sandy soils from the depth of 0.4–1.3 m (B3, C2) (IV). In addition, the comparisons were made between the shallowest (depth 0.3 m, R1) and the deepest (depth 14.2–16.7 m, R3, R4) sandy drilling sediments (railway), and in the clayey drilling sediments (garden) (depth 0.8 m, G1 and 3.5–14.4 m, G2-G4) (II). The differences between groundwater environment and drilling sediments were obtained comparing groundwater deposits (depth 6.3–21.0 m) to the drilling sediments below water table (6.1–24.8 m) (II, III).

The soil type differences between sandy (OM 0.8–3.8 % dry wt, depth 0.1–0.5 m, A1, B5, C2), low organic soil (OM 8.0 % dry wt, depth 0.2–0.3 m, C4, C6), and high organic soil (OM 69.9 % dry wt, depth 0.1–0.3 m, A6) were observed (IV). Moreover, the soil type impacts were monitored in the contaminated sandy (OM

0.8–2.1 % dry wt, G5, G6) and clayey drilling sediments (OM 1.4–2.8 % dry wt, G2-G4) at the depth of 3.5–24.8 m (II).

Atrazine impacts on microbial communities were investigated in groundwater deposits (depth 6.3–21.0 m, pipes A, C, D, and well). Pipe B was excluded due to the bank filtration of lake water, which altered its microbial community composition (III). The effects of DDT pollution on microbial community compositions were examined by comparing the surface soils with three concentrations of DDT and its degradation products to controls (B5, C2, C4, C6) with approximately the same OM content: i) 0.02–0.03 mg kg^{-1} (0.3–0.4 μg g^{-1} OM; B6, B7), ii) 0.07–0.2 mg kg^{-1} (1.4–3.6 μg g^{-1} OM; B2, B4), and iii) 0.2–0.8 mg kg^{-1} (8.4–19.5 μg g^{-1} OM; C3, C5) (IV). The effects of pentachloroaniline (PeCA) and hexachlorobenzene (HCB) contamination on microbial community compositions were observed in soils with PeCA and HCB together 0.3–0.6 mg kg^{-1} (5.3–6.8 μg g^{-1} OM; A3, A5) and in soils with PeCA 0.04–0.06 mg kg^{-1} (1.2–2.7 μg g^{-1} OM; A2, C1) compared to uncontaminated samples (A1, B5, C2, C4, C6) (IV).

3.7 DATA ANALYSIS

To study the responses in the microbial community to depth, soil type, and pesticides, the data sets of the relative content of individual phospholipid (PL), glycolipid (GL), and neutral lipid (NL) FAs were subjected to principal component analysis, PCA (II, III, IV). Then the data sets of all individual FAs were exposed to two factor non-parametric Kruskal-Wallis (K-W) test, and Mann-Whitney (M-W) test to find statistically-significant differences. The Pearson two-tailed correlation coefficient was used to relate soil and deposit parameters to the individual FAs and calculated indices of lipid profiles.

4 RESULTS AND DISCUSSION

4.1 DEPTH-DEPENDENT CHANGES IN MICROBIAL LIPIDS IN VARIOUS SOILS

Depth-dependent changes were found in microbial biomass (lipid quantity; cell numbers) and microbial community composition (lipid profiles). Microbial biomass declined in deeper layers compared to the deposits near soil surface, independent of pesticide contamination. The variation in lipid quantities was the greatest in the topsoil layers, and less in the deeper sediments. The quantity of PLFAs ranged from 5–228 μg g^{-1} (depth 0.1–0.3 m) to 1–2 μg g^{-1} (depth 3.5–24.8 m) (II, IV). As in PLFAs, similar reductions with depth were also seen in the quantities of GLFAs (from 6–185 μg g^{-1} to 0.4–3 μg g^{-1}) and NLFAs (from 8–683 μg g^{-1} to 1–9 μg g^{-1}). Microbial cell numbers, which were calculated from the quantity of PLFAs, reduced with increasing depth from 7 x 10^8 cells g^{-1} to 3 x 10^7 cells g^{-1}. The decrease in the PLFA quantity with soil depth has been reported by many authors, while there is little information concerning the quantities of GLFAs and NLFAs (Balkwill et al. 1997, Taylor et al. 2002, Allison et al. 2007).

Three different PLFA profiles were found with increasing depth (II, IV). The depth dependent changes of GLFA and NLFA profiles resembled those of PLFA profiles (Table 2 a, b, c, II, IV). First, PLFAs extracted from surface soils at the depths of about 0.1–0.3 m, showed the highest percentages of monounsaturated straight-chain FAs common for Gram-negative bacteria, and polyunsaturated FAs characteristic of fungi (Table 2 a) (Zelles 1999). Further, the percentages of cyclopropane FAs were highest in pristine soil at the depths of 0.1–1.3 m, signifying Gram-negative bacteria and lactobacilli or slow growth (Bossio and Scow, 1998). Second, below the surface at the depths of 0.4–1.3 m, the proportions of branched-chain FAs, including *iso*- and *anteiso*-branched (Gram-positive bacteria), TBSA (actinobacteria), and 10-Me-16:0 (SRB), were increased in PLFAs or remained at the same level as in surface soils, and below the depth of 1.3 m their percentage declined (Table 2 a). The low abundance of fungi at the depth of 0.4–1.3 m could be understood through the sensitivity of fungi to annual freeze and thaw-cycles in soil (Feng et al. 2007, Schmitt et al. 2008). Other explanations may include: low fungal abundance in grassland or non-vegetated soil, and greater depth (Bardgett 2005, Holden and Fierer 2005). Third, in the sediments below 1.3 m, the high percentages of the saturated straight-chain FAs suggested slow growth in microbial communities. Interestingly, in the deepest drilling sediments, the lowest values of cyclopropane FAs, 10-Me-16:0, and TBSA were detected. These results are essentially consistent

with previous studies (Holden and Fierer 2005, Allison et al. 2007, Feng et al. 2007, Högberg et al. 2007, Bach et al. 2008).

Microbial cells have survived in stationary growth phase better than in exponential growth phase, due to the more rigid stationary cell membrane; therefore this state promotes microbial survival in deep sediments (Muñoz-Rojas et al. 2006). Indeed, low ratios of unsaturated to saturated FAs, as well as high percentages of the saturated straight-chain FAs and high average C numbers (FA chain length) in PLFAs, indicated low microbial activity and a high portion of stationary growth phase cells in the deepest drilling sediments. In addition, these indices seemed to be most useful to describe stressful conditions in deep drilling sediments, as the percentages of *trans*-monounsaturated and cyclopropane FAs were extremely low and unreliable for evaluating stress (Table 2 a, b, c, II). In GLFAs the saturated straight-chain FAs were the highest biomarker group in all soil samples, comprising about 50% or more of the FAs, reflecting the general amount of GLFAs more significantly than slow growth. The results of this study showed that the microbial activity seemed to differ between old abandoned market garden soils and the shallowest drilling sediments. The ratios of unsat/sat were slightly lower and the average C numbers higher in clean market garden soils at the depths of 0.1–1.3 m than in railway and garden sediments at the depths of 0.3–0.8 m. This may be related to the lower nutrient load in old market garden soils in comparison to the layers next to railway or clay layers below the garden. Microbial activity has been reported to reduce with agricultural soil abandonment, which seemed to also be true in old market garden soils (García et al.1997, Lõhmus et al. 2005).

Table 2 a, b, c. The percentages of main FA groups and calculated indices of PLFAs (2a), GLFAs (2b), and NLFAs (2c) in surface soils, drilling sediments, and groundwater deposits at different depths. Superscript letters indicate statistically-significant differences between samples (MW≤0.05, a, f, g; s.d. b, c, d ,e).

2 a.

	Surface soils (market gardens)		Drilling sediments*(railway)		Drilling sediments*(garden)		Groundwater deposits**
Depth m; (OM % dry wt)	0.1-0.3; (0.8-3.8)	0.4-1.3; (2.6-4.8)	0.3; (2.3)	14.2-16.7; (0.4-0.5)	0.8; (1.5)	3.5-14.4; (1.4-2.8)	6.3-19.9; (1.4-2.5)
Sum of biomarker fatty acids (%), physiological indices							
PLFA							
Monounsaturated (16: 1ω9c,16: 1ω7c,16: 1ω5c, 18:1 ω9c, 18:1 ω7c, 16: 1ω5c)	**28.9 ± 2.2**^{a,e}	**18.0 ± 1.1**^{a,b}	**44.7 ± 1.7**^{b,c,d}	**11.3 ± 2.7**^{d}	**22.5 ± 1.4**^{c,e}	**25.1 ± 0.9**^{g}	**44.2 ± 2.6**^{g}
Polyunsaturated (18:2ω6,9)	**2.3 ± 0.3**^{a,e}	**1.0 ± 0.2**^{a,b}	**2.5 ± 0.6**^{b,c,d}	**1.0 ± 0.1**^{d}	**1.0 ± 0.7**^{c,e,f}	**2.1 ± 0.2**^{f,g}	**1.0 ± 0.5**^{g}
Iso-anteiso-branched-chain (i-14:0, i-15:0, a-15:0, i-16:0, i-17:0, a-17:0, i-18:0)	**20.5 ± 0.5**^{e}	20.3 ± 0.5	**20.3 ± 0.1**^{c,d}	**3.5 ± 1.4**^{d}	**33.8 ± 0.5**^{c,e,f}	**8.6 ± 0.3**^{f,g}	**11.3 ± 1.5**^{g}
i/a-15:0	1.5 ± 0.1	**1.6 ± 0.1**^{b}	**0.6 ± 0.0**^{b,c}	0.7 ± 0.1	**1.2 ± 0.2**^{c,f}	**0.4 ± 0.0**^{f,g}	**1.4 ± 0.1**^{g}
i/a17:0	1.2 ± 0.1	1.3 ± 0.1	**1.4 ± 0.1**^{d}	**0.6 ± 0.1**^{d}	1.2 ± 0.1	1.3 ± 0.2	1.4 ± 0.2
Sum of branched-chain (iso-, anteiso-,middle-, br-15:0a,b,c, br-17:0a,b, br18:0a,b, br-19:0a,b)	**34.9 ± 1.1**^{e}	**38.4 ± 0.5**^{b}	**28.2 ± 0.2**^{b,c,d}	**5.5 ± 2.0**^{d}	**56.4 ± 1.0**^{c,e,f}	**16.2 ± 0.6**^{f}	19.8 ± 1.1
Saturated straight-chain (14:0,16:0,17:0,18:0, 20:0)	**24.9 ± 0.3**^{a}	**35.4 ± 2.0**^{a,b}	**23.3 ± 1.4**^{b,c,d}	**81.7 ± 5.1**^{d}	**19.1 ± 0.2**^{c,f}	**55.4 ± 0.9**^{f,g}	**33.2 ± 2.8**^{g}
Cyclopropane fatty acids (cy-17:0ω9, cy-17:0ω7, cy-19:0ω7)	**9.1 ± 2.2**^{e}	**7.3 ± 1.6**^{b}	**1.3 ± 0.1**^{b,d}	**0.5 ± 0.2**^{d}	**1.0 ± 0.2**^{e}	1.2 ± 0.6	1.8 ± 0.5
cy-17:0/pre	0.3 ± 0.0	**0.5 ± 0.1**^{b}	**0.1 ± 0.0**^{b}	0.3 ± 0.2	0.2 ± 0.0	**0.2 ± 0.1**^{g}	**1.0 ± 0.0**^{g}
cy-19:0/pre	0.4 ± 0.1	0.5 ± 0.2	0.1 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	**0.1 ± 0.1**^{g}	**1.0 ± 0.0**^{g}
10Me16:0	**5.4 ± 0.5**^{a,e}	**8.7 ± 1.5**^{a,b}	**2.4 ± 0.9**^{b,c,d}	**0.6 ± 0.2**^{d}	**12.6 ± 0.3**^{c,e,f}	**1.8 ± 0.5**^{f}	2.3 ± 0.4
TBSA	**1.1 ± 0.1**^{e}	**1.0 ± 0.3**	**0.6 ± 0.0**^{c,d}	**0.2 ± 0.2**^{d}	**2.6 ± 0.8**^{c,e,f}	**0.4 ± 0.2**^{f}	0.4 ± 0.2
Unsat/Sat	0.5 ± 0.1	**0.3 ± 0.0**^{b}	**0.9 ± 0.0**^{b,c,d}	**0.1 ± 0.0**^{d}	**0.3 ± 0.0**^{c}	**0.4 ± 0.0**^{g}	**1.2 ± 0.1**^{g}
Average carbon number	**283.1 ± 1.2**^{e}	281.0 ± 0.6	**279.9 ± 0.2**^{c,d}	**283.9 ± 1.0**^{d}	**277.9 ± 0.4**^{c,e,f}	**280.6 ± 0.5**^{f}	277.8 ± 1.2

*) atrazine 12.2-14.0 μg kg^{-1}, simazine 17.7-34.8 μg kg^{-1}, BAM 5.3-32.4 μg kg^{-1}

**) atrazine 0.03-0.36 μg l^{-1}, DEA 0.09-0.20 μg l^{-1}, DIA 0-0.04 μg l^{-1}

2 b.

	Surface soils (market gardens)		Drilling sediments*(railway)		Drilling sediments*(garden)		Groundwater deposits**
Depth m; (OM % dry wt)	0.1-0.3; (0.8-3.8)	0.4-1.3;(2.6-4.8)	0.3; (2.3)	14.2-16.7; (0.4-0.5)	0.8; (1.5)	3.5-14.4; (1.4-2.8)	6.3-19.9; (1.4-2.5)
Sum of biomarker fatty acids (%), physiological indices							
GLFA							
Monounsaturated (16: 1ω9c,16: 1ω7c,16: 1ω5c, 18:1 ω9c, 18:1 ω7c, 16: 1ω5c)	**12.0 ± 1.4^{e}**	**7.0 ± 0.8^{b}**	**21.2 ± 1.8^{b,d}**	**11.9 ± 4.5^{d}**	**21.9 ± 2.3^{e}**	**19.9 ± 3.6^{g}**	**57.6 ± 10.5^{g}**
Polyunsaturated (18:2ω6,9)	1.2 ± 0.7	**0.6 ± 0.3^{b}**	**3.2 ± 0.8^{b,c,d}**	**0.4 ± 0.3^{d}**	**0.7 ± 0.5^{c}**	1.6 ± 0.1	0.7 ± 0.1
Iso-anteiso-branched-chain (i-14:0, i-15:0, a-15:0, i-16:0, i-17:0, a-17:0, i-18:0)	11.9 ± 1.6	**8.5 ± 0.9^{b}**	**21.1 ± 0.1^{b,c,d}**	**3.4 ± 0.8^{d}**	**17.9 ± 0.7^{c,f}**	**7.9 ± 0.1^{f}**	5.9 ± 0.7
i/a-15:0	0.7 ± 0.0	0.8 ± 0.1	0.8 ± 0.0	0.8 ± 0.4	0.8 ± 0.1	**0.8 ± 0.0^{g}**	**1.4 ± 0.1^{g}**
i/a17:0	**0.6 ± 0.0^{e}**	0.7 ± 0.0	**0.9 ± 0.0^{c,d}**	**0.4 ± 0.1^{d}**	**1.8 ± 0.2^{c,e,f}**	**0.4 ± 0.0^{f}**	0.4 ± 0.1
Sum of branched-chain (iso-, anteiso-,middle-, br-15:0a,b,c, br-17:0a,b, br18:0a,b, br-19:0a,b)	**19.1 ± 3.4^{e}**	**14.4 ± 1.4^{b}**	**23.6 ± 0.2^{b,c,d}**	**3.9 ± 0.7^{d}**	**26.3 ± 0.8^{c,e,f}**	**8.7 ± 0.2^{f}**	8.2 ± 3.5
Saturated straight-chain (14:0,16:0,17:0,18:0, 20:0)	**51.8 ± 0.9^{a}**	**72.8 ± 2.5^{a,b}**	**47.8 ± 0.9^{b,d}**	**83.8 ± 4.3^{d}**	**49.8 ± 3.2^{f}**	**69.4 ± 2.8^{f,g}**	**32.9 ± 8.0^{g}**
Cyclopropane fatty acids (cy-17:0ω9, cy-17:0ω7, cy-19:0ω7)	**15.9 ± 4.2^{e}**	5.2 ± 2.6	**4.2 ± 0.1^{c,d}**	**tr^{d}**	**1.3 ± 0.5^{c,e,f}**	**0.4 ± 0.1^{f}**	0.6 ± 0.0
cy-17:0/pre	**2.3 ± 1.3^{e}**	3.3 ± 3.1	0.4 ± 0.0	-	**0.5 ± 0.3^{e}**	0.4 ± 0.0	0.2 ± 0.1
cy-19:0/pre	**2.4 ± 0.8^{e}**	0.8 ± 0.4	**0.5 ± 0.0^{c}**	-	**0.1 ± 0.0^{c,e}**	0.0 ± 0.0	0.0 ± 0.0
10Me16:0	**1.2 ± 0.1^{e}**	1.0 ± 0.1	**1.2 ± 0.0^{c,d}**	**tr^{d}**	**3.8 ± 0.2^{c,e,f}**	**0.2 ± 0.1^{f}**	tr
TBSA	**0.4 ± 0.3^{e}**	0.5 ± 0.2	**0.4 ± 0.0^{c,d}**	**n.d.^{d}**	**3.0 ± 0.1^{c,e,f}**	**0.3 ± 0.0^{f}**	n.d.
Unsat/Sat	0.2 ± 0.0	**0.1 ± 0.0^{b}**	**0.4 ± 0.0^{b,d}**	**0.1 ± 0.1^{d}**	0.3 ± 0.1	**0.3 ± 0.1^{g}**	**1.4 ± 0.6^{g}**
Average carbon number	286.3 ± 0.2	**282.5 ± 0.5^{b}**	**277.7 ± 0.2^{b,c,d}**	**282.3 ± 1.3^{c,d}**	**281.1 ± 0.2^{c,f}**	**283.1 ± 0.4^{f,g}**	**287.9 ± 0.5^{g}**

*) atrazine 12.2-14.0 μg kg^{-1}, simazine 17.7- 34.8 μg kg^{-1}, BAM 5.3-32.4 μg kg^{-1}
**) atrazine 0.03-0.36 μg l^{-1}, DEA 0.09-0.20 μg l^{-1}, DIA 0- 0.04 μg l^{-1}
n.d., not detected; tr, trace amounts

2 c.

	Surface soils (market gardens)		Subsurface soils (railway sediments)		Subsurface soils (garden sediments)		Groundwater deposits**
Depth (m); OM % dry wt	0.1-0.3; (0.8-3.8)	0.4-1.3; 2.6-4.8	0.3; 2.3	14.2-16.7; 0.4-0.5	0.8; 1.5	3.5-14.4; 1.4-2.8	6.3-19.9; (1.4-2.5)
Sum of biomarker fatty acids (%), physiological indices							
NLFA							
Monounsaturated (16: 1ω9c,16: 1ω7c,16: 1ω5c, 18:1 ω9c, 18:1 ω7c, 16: 1ω5c)	**34.1 ± 2.7**^{a,e}	**21.6 ± 0.9**^{a,b}	**45.2 ± 4.5**^{b,d}	**26.4 ± 6.1**^{d}	**45.9 ± 0.5**^{e,f}	**25.9 ± 5.7**^{f,g}	**70.8 ± 2.8**^{g}
Polyunsaturated (18:2ω6,9)	**3.5 ± 0.0**^{e}	**3.2 ± 0.7**^{b}	**12.5 ± 2.6**^{b,c,d}	**2.2 ± 0.2**^{d}	**2.8 ± 0.1**^{c,e}	2.8 ± 0.5	1.6 ± 0.4
Iso-anteiso-branched-chain (i-14:0, i-15:0, a-15:0, i-16:0, i-17:0, a-17:0, i-18:0)	7.8 ± 0.4	**6.5 ± 0.7**^{b}	**8.5 ± 0.5**^{b,d}	**3.7 ± 0.8**^{d}	**9.0 ± 1.1**^{f}	**3.5 ± 0.1**^{f}	2.7 ± 0.2
i/a-15:0	1.0 ± 0.2	1.1 ± 0.1	**0.7 ± 0.1**^{c}	0.8 ± 0.4	**1.4 ± 0.1**^{c,f}	**0.7 ± 0.0**^{f}	0.9 ± 0.1
i/a17:0	**0.5 ± 0.0**^{e}	**0.6 ± 0.1**	**0.8 ± 0.0**^{d}	**0.4 ± 0.1**^{d}	**1.1 ± 0.1**^{e,f}	**0.4 ± 0.0**^{f}	0.4 ± 0.0
Sum of branched-chain (iso-, anteiso-,middle-, br-15:0a,b,c, br-17:0a,b, br18:0a,b, br-19:0a,b)	**15.2 ± 1.5**^{a}	**10.8 ± 1.4**^{a}	**10.4 ± 0.4**^{c,d}	**4.4 ± 0.7**^{d}	**14.0 ± 1.4**^{c,f}	**4.0 ± 0.2**^{f}	6.1 ± 0.8
Saturated straight-chain (14:0,16:0,17:0,18:0, 20:0)	**40.7 ± 2.4**^{a}	**62.6 ± 1.4**^{a,b}	**31.4 ± 1.4**^{b,c,d}	**67.0 ± 6.0**^{d}	**37.1 ± 2.0**^{c,f}	**67.3 ± 4.9**^{f,g}	**22.8 ± 2.7**^{g}
Cyclopropane fatty acids (cy-17:0ω9, cy-17:0ω7, cy-19:0ω7)	**6.5 ± 4.9**^{e}	**1.8 ± 1.0**^{b}	**0.5 ± 0.0**^{b,c,d}	**tr**^{d}	**0.2 ± 0.0**^{c,e}	**tr**^{g}	**0.7 ± 0.0**^{g}
cy-17:0/pre	0.4 ± 0.3	**0.4 ± 0.2**^{b}	**0.0 ± 0.0**^{b}	-	0.0 ± 0.0	-	0.1 ± 0.0
cy-19:0/pre	0.3 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	-	0.0 ± 0.0	-	0.0 ± 0.0
10Me16:0	**1.6 ± 0.5**^{e}	1.0 ± 0.1	**0.7 ± 0.0**^{c,d}	**0.2 ± 0.1**^{d}	**3.6 ± 0.1**^{c,e,f}	**0.2 ± 0.1**^{f}	0.2 ± 0.0
TBSA	0.4 ± 0.1	0.2 ± 0.0	**tr**^{c}	tr	**0.6 ± 0.1**^{c,f}	**n.d.**^{f}	n.d.
Unsat/Sat	**0.8 ± 0.1**^{a}	**0.4 ± 0.0**^{a,b}	**1.4 ± 0.1**^{b,c,d}	**0.4 ± 0.1**^{d}	**1.0 ± 0.0**^{c,f}	**0.4 ± 0.1**^{f,g}	**2.5 ± 0.3**^{g}
Average carbon number	**281.8 ± 0.5**^{e}	**282.2 ± 0.4**^{b}	**277.2 ± 0.2**^{b,c,d}	**284.6 ± 1.0**^{d}	**278.7 ± 0.1**^{c,e,f}	**283.3 ± 0.9**^{f}	287.8 ± 0.4

*) atrazine 12.2-14.0 μg kg^{-1}, simazine 17.7- 34.8 μg kg^{-1}, BAM 5.3-32.4 μg kg^{-1}
**) atrazine 0.03-0.36 μg l^{-1}, DEA 0.09-0.20 μg l^{-1}, DIA 0- 0.04 μg l^{-1}
n.d., not detected; tr, trace amounts

4.2 DIFFERENCES IN FATTY ACID PROFILES BETWEEN DRILLING SEDIMENTS AND GROUNDWATER DEPOSITS

The quantities of lipids in the groundwater deposits from pipes and well were generally higher than in garden drilling sediments (II, III) despite the same approximate depth (Fig. 1). Consequently, the estimated microbial cell numbers in groundwater deposits were greater than in drilling sediments at the respective depths below the water table. In the deepest drilling sediments and groundwater deposits, cell numbers were at the same magnitude (circa 7 x 10^7 cells g^{-1}) and comparable with earlier data of drinking water and lake sediments (circa 3 x 10^7 cells g^{-1}) (Keinänen et al. 2003).

The quantity of GLFAs was commonly equal or higher than that of PLFAs in groundwater deposits, while in the drilling sediments of garden, the quantities of PLFAs were higher than those of GLFAs in all clayey layers of garden sediments down to the depth of 14.4 m. The increased occurrence of GLFAs in groundwater deposits and some drilling sediments could be explained by phosphorous limitation in groundwater deposits, resulting in the replacement of PLFAs with GLFAs (II, III, Miettinen et al.1996). In addition, the growth of Gram-positive bacteria might have caused the enhancement of GLFAs (Kontro et al. 2006). The quantities of NLFAs were equal or higher than those of PLFAs and GLFAs in groundwater deposits and drilling sediments, which is possibly due to the occurrence of carbon excess or higher proportion of dead cells (Bååth, 2003, II).

These results showed that the proportions of Gram-negative bacteria indicating monounsaturated acids and Gram-positive bacteria indicating *iso*- and *anteiso*-branched acids in groundwater deposits were higher than in drilling sediments, while the trend was opposite in the percentages of the saturated straight-chain FAs in PLFAs, GLFAs, and NLFAs. The explanation for these results could be that the percolated substrates were available for microbes and a greater range of resources affected higher proportions of Gram-negatives and -positives in groundwater deposits (John and Rose 2005).

In PLFAs, the higher ratios of unsaturated to saturated FAs and lower average C numbers in groundwater deposits than in drilling sediments indicated greater microbial activity in groundwater deposits compared to drilling sediments (Table 2 a). For GLFAs, however, greater microbial activity could only be seen in the high ratios of unsat/sat (Table 2 b). Moreover, the PLFA profile of the groundwater deposits was closer to that of the shallowest (depth 0.3 m) railway sediment than to the profiles of drilling sediments at the respective depths. However, this similarity could not been found in GLFAs and NLFAs (Table 2 a, b, c).

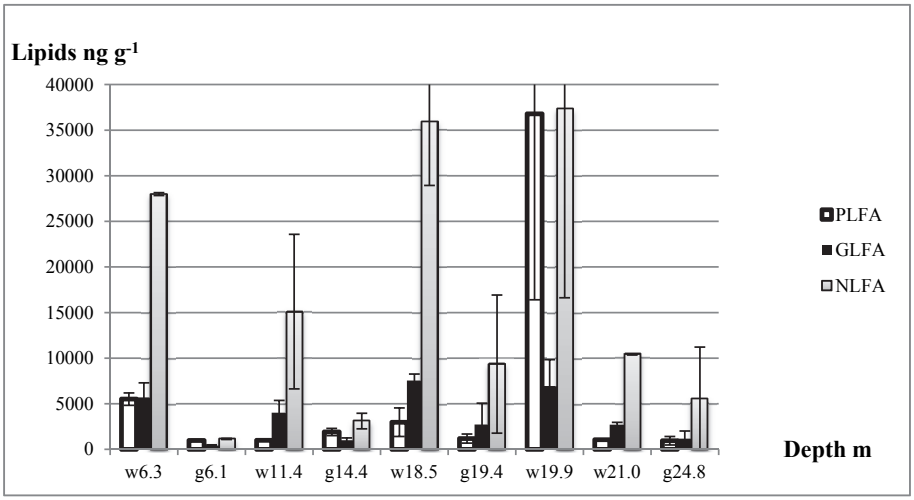

Fig. 1. PLFA, GLFA, and NLFA quantities (n gg^{-1}) in garden sediments (g) and groundwater deposits (w).

4.3 SOIL TYPE EFFECTS ON MICROBIAL COMMUNITIES

Soil OM content has been reported to be the main factor influencing microbial biomass in soil (Sylvia et al. 2005, Fierer et al. 2009), which was found to also be true in this study. In surface soils, the quantities of PLFAs were equal or higher than those of GLFAs, and equal or lower than those of NLFAs (IV). The quantity of PLFAs varied between 5 to 43 μg g^{-1} in sand, between 40 to 45 μg g^{-1} in low organic soils, and it was 228 μg g^{-1} in high organic soil. The quantities of GLFAs ranged from 4 μg g^{-1} to 186 μg g^{-1}, and the quantities of NLFAs from 8 μg g^{-1} to 683 μg g^{-1} (Fig 2 A). In drilling sediments of garden, the quantities of microbial lipids in clayey sediments were of the same order of magnitude as in sandy sediments, being 1–2 μg g^{-1} in PLFAs, 0.4–3 μg g^{-1} in GLFAs, and 1–9 μg g^{-1} in NLFAs (Fig 2 B). In soil layers at the depths of 0.1–4.2 m and at 4.5–31.3 m, microbial biomass has been shown to be lower in sand than in clay (Albrechtsen and Winding 1992, Taylor et al. 2002, Bach et al. 2010). The opposite results of our study can be explained by the fact that in deep drilling sediments, below garden nutrients flowed with groundwater and improved conditions for microbes. Microbial cell numbers extended to 2×10^8 cells g^{-1} in clayey and 8×10^7 cells g^{-1} in sandy sediments (II).

When pristine surface soils were compared, the effects of soil type, sand and organic, were obtained. In PLFAs, GLFAs, and NLFAs, the elevated percentages of cyclopropane FAs, biomarkers for Gram-negative and lactic acid bacteria, were related to soil with the high OM content (Table 3 a,b,c). Especially in GLFAs and NLFAs, the most prominent difference between organic and sandy soil was the

huge abundance of cyclopropane FAs in the high organic soil, which was possibly unrelated to stressful conditions (Bossio and Scow 1998, IV). Stress indicators such as the ratio of unsat/sat and average C numbers in PLFAs were of nearly the same magnitude in organic and sandy soils as well as the ratios of cy/pre in PLFAs were low, and consequently no stress due to soil type was found. In PLFAs, the highest percentages of the monounsaturated and polyunsaturated FAs in high organic soil indicated Gram-negative bacteria and fungi, respectively, which has also been reported by other researchers (Bardgett et al. 1999, Keith-Roach et al. 2002, Habekost et al. 2008). In PLFAs, the portion of *iso*- and *anteiso*-branched acids from Gram-positive bacteria was higher in low organic soils than in the high organic soil, showing that Gram-positive bacteria typically grow with lower supply of nutritional resources than Gram-negatives (van Elsas et al. 2007). In sandy soils, there were the greatest percentages of 10-Me-16:0 from SRB and TBSA from actinobacteria in PLFAs, and in NLFAs the sum of branched-chain FAs (including TBSA and 10-Me-16:0) was higher than in organic soils, which is in accordance with previous studies (Zak et al 2003). Overall, more soil type effects were found in PLFAs and NLFAs than in GLFAs.

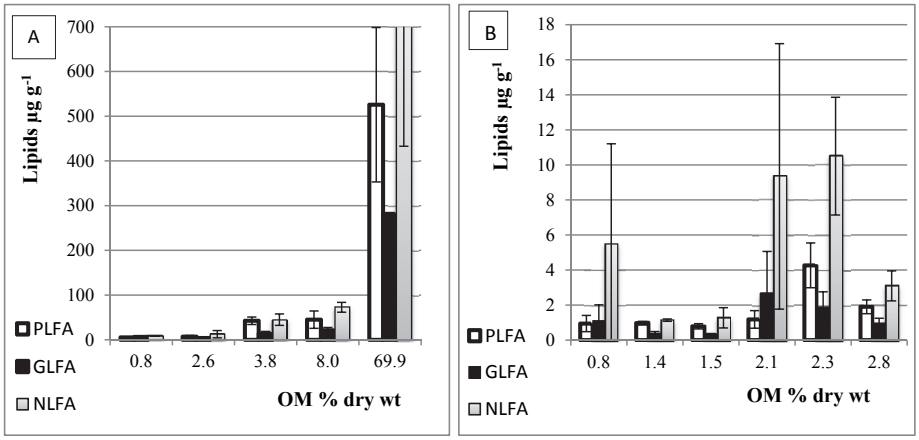

Fig. 2. The quantity of PLFA, GLFA, and NLFA in pristine surface soil (old market gardens), OM content 0.8–69.9 % dry wt (A) and atrazine, simazine, BAM contaminated subsurface soil deposits (B) with OM content 0.8–2.8 % dry wt.

In drilling sediments, soil type, sand and clay, had more effects on microbial community composition than water table (II). In PLFAs, GLFAs, and NLFAs there were higher proportions of branched-chain FAs, especially *iso*- and *anteiso*-branched acids (Gram-positive bacteria) in clay than in sandy layers of garden sediments (Table 3 a, b, c). In PLFAs and GLFAs, the percentage of 10-Me-16:0 (SRB) was also higher in clay than in sand. These FA profiles seemed to be related

anaerobic clayey sediment layers (II). Microbial activity, observed via the ratios of unsat/sat and average C numbers in PLFAs and GLFAs, did not differ between the soil types in drilling sediments (3 a, b). In NLFAs, the main difference between deep clayey and sandy drilling sediments was the high abundance of the saturated straight-chain FAs in clayey sediments; while the percentage of monounsaturated straight-chain acids was greater in sandy sediments (Table 3 c). Nevertheless, there were only few significant differences between the deep clayey and sandy layers in NLFAs in drilling sediments, indicating the weakest selection pressure for the NLFA composition.

Table 3 a, b, c. The percentages of main PLFA (3a), GLFA (3b), and NLFA (3c) biomarker FA groups and calculated indices in high organic (OM 69.9 % dry wt), low organic (OM 8.0 % dry wt), and sandy (OM 2.6-3.8 % dry wt) surface soils, and in clayey (OM 1.4-2.8 % dry wt) and sandy (OM 0.8-2.1 % dry wt) drilling sediments. Letters in superscripts indicate statistically-significant differences between samples (s.d. a, b; MW≤0.05, c, d).

3 a.

	Surface soils (organic)		**Surface soils (sand)**	**Drilling sediments*(clay)**	**Drilling sediments*(sand)**
Depth m; (OM % dry wt)	0.1-0.3; (69.9)	0.2-0.3; (8.0)	0.1-0.5; (0.8-3.8)	3.5-14.4; (1.4-2.8)	19.4-24.8; (0.8-2.1)
Sum of biomarker fatty acids (%),					
physiological indices					
PLFA					
Monounsaturated**	**31.3 ± 1.1**^{b}	**20.4 ± 0.5**^{b}	25.2 ± 1.1	25.1 ± 0.9	16.7 ± 8.7
(16: 1ω9c,16: 1ω7c,16: 1ω5c,					
18:1 ω9c, 18:1 ω7c, 16: 1ω5c)					
Polyunsaturated	**2.8 ± 0.0**^{a,b}	**1.8 ± 0.1**^{b}	**1.9 ± 0.2**^{a}	2.1 ± 0.2	2.1 ± 0.3
(18:2ω6,9)					
Iso-,anteiso-branched-chain***	**21.8 ± 1.4**^{b}	**25.1 ± 0.4**^{b,c}	**20.3 ± 0.3**^{c}	**8.6 ± 0.3**^{d}	**3.8 ± 0.7**^{d}
(i-14:0, i-15:0, a-15:0,					
i-16:0, i-17:0, a-17:0, i-18:0)					
i/a-15:0	**2.7 ± 0.3**^{a}	2.3 ± 0.0	**1.9 ± 0.1**^{a}	**0.4 ± 0.0**^{d}	**0.6 ± 0.0**^{d}
i/a17:0	**1.5 ± 0.2**^{b}	**2.5 ± 0.1**^{b,c}	**1.5 ± 0.1**^{c}	**1.3 ± 0.2**^{d}	**0.6 ± 0.1**^{d}
Sum of branched	37.4 ± 0.4	40.4 ± 0.4	36.6 ± 1.3	**16.2 ± 0.6**^{d}	**7.4 ± 1.2**^{d}
(iso-, anteiso-,middle-, br-15:0a,b,c,					
br-17:0a,b, br18:0a,b, br-19:0a,b)					
Saturated straight-chain	**15.0 ± 1.5**^{a,b}	**27.4 ± 1.1**^{b}	**28.1 ± 0.8**^{a}	55.4 ± 0.9	73.0 ± 8.2
(14:0,16:0,17:0,18:0, 20:0)					
Cyclopropane fatty acids	**13.5 ± 0.7**^{a,b}	**10.4 ± 0.7**^{b}	**9.7 ± 1.6**^{a}	1.2 ± 0.6	0.8 ± 0.4
(cy-17:0ω9, cy-17:0ω7, cy-19:0ω7)					
cy-17:0/pre	**0.2 ± 0.0**^{b}	**0.4 ± 0.0**^{b}	0.4 ± 0.0	0.2 ± 0.1	0.3 ± 0.2
cy-19:0/pre	0.7 ± 0.1	0.8 ± 0.0	0.6 ± 0.2	0.1 ± 0.1	0.1 ± 0.0
10Me16:0	**5.5. ± 0.4**^{b}	**4.3 ± 0.2**^{b,c}	**5.9 ± 0.4**^{c}	**1.8 ± 0.5**^{d}	**0.6 ± 0.2**^{d}
TBSA	**0.8 ± 0.0**^{a}	**1.0 ± 0.1**	**1.2 ± 0.1**^{a}	0.4 ± 0.2	0.2 ± 0.1
Unsat/Sat	**0.7 ± 0.1**^{b}	**0.3 ± 0.0**^{b}	0.4 ± 0.0	0.4 ± 0.0	0.2 ± 0.2
Average carbon number	**284.2 ± 0.9**^{b}	**282.6 ± 0.3**^{b}	282.8 ± 0.8	280.6 ± 0.5	281.8 ± 0.1

* atrazine 12.2-14.0 μg kg^{-1}, simazine 17.7- 34.8 μg kg^{-1}, BAM 5.3-32.4 μg kg^{-1}

** in surface soils 18:1μ9c excluded

*** in surface soils i-19:0 is included

3 b.

	Surface soils (organic)		Surface soils (sand)	Drilling sediments* (clay)	Drilling sediments* (sand)
Depth m; (OM % dry wt)	0.1-0.3; (69.9)	0.2-0.3; (8.0)	0.1-0.5; (0.8-3.8)	3.5-14.4; (1.4-2.8)	19.4-24.8; (0.8-2.1)
Sum of biomarker fatty acids (%),					
physiological indices					
GLFA					
Monounsaturated**	5.4 ± 0.3	5.0 ± 0.3	9.8 ± 0.8	19.9 ± 3.6	30.0 ± 3.6
(16: 1ω9c,16: 1ω7c,16: 1ω5c,					
18:1 ω9c, 18:1 ω7c, 16: 1ω5c)					
Polyunsaturated	**0.4 ± 0.1**^{b}	**1.3 ± 0.2**^{b}	0.9 ± 0.3	**1.6 ± 0.1**^{d}	**0.6 ± 0.2**^{d}
(18:2ω6,9)					
Iso-anteiso-branched-chain***	**6.3 ± 0.1**^{a,b}	**9.5 ± 0.1**^{b}	**11.1 ± 1.0**^{a}	**7.9 ± 0.1**^{d}	**4.0 ± 0.7**^{d}
(i-14:0, i-15:0, a-15:0,					
i-16:0, i-17:0, a-17:0, i-18:0)					
i/a-15:0	**1.8 ± 0.1**^{a}	**1.5 ± 0.1**^{c}	**0.7 ± 0.1**^{a,c}	0.8 ± 0.0	0.6 ± 0.0
i/a17:0	**0.3 ± 0.0**^{a,b}	**1.6 ± 0.1**^{b,c}	**0.7 ± 0.0**^{a,c}	0.4 ± 0.0	0.3 ± 0.0
Sum of branched-chain	**8.6 ± 0.2**^{a,b}	**13.5 ± 0.1**^{b}	**17.3 ± 2.9**^{a}	**8.7 ± 0.2**^{d}	**4.4 ± 0.8**^{d}
(iso-, anteiso-,middle-, br-15:0a,b,c,					
br-17:0a,b, br18:0a,b, br-19:0a,b)					
Saturated straight-chain	**22.0 ± 0.6**^{a,b}	**60.4 ± 1.1**^{b}	**58.9 ± 1.0**^{a}	69.4 ± 2.8	64.8 ± 4.3
(14:0,16:0,17:0,18:0, 20:0)					
Cyclopropane fatty acids	**63.6 ± 1.0**^{a,b}	**19.8 ± 0.9**^{b}	**13.1 ± 3.1**^{a}	0.4 ± 0.1	0.2 ± 0.1
(cy-17:0ω9, cy-17:0ω7, cy-19:0ω7)					
cy-17:0/pre	**20.0 ± 1.2**^{a,b}	**3.7 ± 0.5**^{b}	**3.8 ± 2.6**^{a}	0.4 ± 0.0	0.3 ± 0.0
cy-19:0/pre	**11.5 ± 1.2**^{a,b}	**5.4 ± 0.1**^{b,c}	**2.5 ± 0.7**^{a,c}	0.0 ± 0.0	0.0 ± 0.0
10Me16:0	0.6 ± 0.0	0.8 ± 0.1	1.0 ± 0.1	**0.2 ± 0.1**^{d}	**tr**^{d}
TBSA	**0.1 ± 0.0**^{a,b}	**0.4 ± 0.1**^{b}	**0.5 ± 0.2**^{a}	**0.3 ± 0.0**^{d}	**n.d.**^{d}
Unsat/Sat	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.5 ± 0.1
Average carbon number	**296.3 ± 0.3**^{a,b}	**287.5 ± 0.5**^{b}	**285.1 ± 0.2**^{a}	283.1 ± 0.4	285.7 ± 0.7

* atrazine 12.2-14.0 μg kg^{-1}, simazine 17.7- 34.8 μg kg^{-1}, BAM 5.3-32.4 μg kg^{-1}
** in surface soils 18:1μ9c excluded
*** in surface soils i-19:0 is included

3 c.

	Surface soils (organic)		Surface soils (sand)	Drilling sediments* (clay)	Drilling sediments*(sand)
Depth m; (OM % dry wt)	0.1-0.3; (69.9)	0.2-0.3; (8.0)	0.1-0.5; (0.8-3.8)	3.5-14.4; (1.4-2.8)	19.4-24.8; (0.8-2.1)
Sum of biomarker fatty acids (%), physiological indices					
NLFA					
Monounsaturated** (16: 1ω9c,16: 1ω7c,16: 1ω5c, 18:1 ω9c, 18:1 ω7c, 16: 1ω5c)	**11.6 ± 0.1**[a]	14.0 ± 0.9	**27.3 ± 1.6**[a]	25.9 ± 5.7	48.0 ± 11.9
Polyunsaturated (18:2ω6,9)	**1.5 ± 0.2**[a,b]	**2.4 ± 0.3**[b]	**3.3 ± 0.0**[a,b]	2.8 ± 0.5	2.1 ± 0.4
Iso-anteiso-branched-chain*** (i-14:0, i-15:0, a-15:0, i-16:0, i-17:0, a-17:0, i-18:0)	6.0 ± 0.0	**5.1 ± 0.5**[c]	**7.3 ± 0.5**[c]	**3.5 ± 0.1**[d]	**2.8 ± 0.2**[d]
i/a-15:0	1.2 ± 0.4	1.8 ± 0.1	1.1 ± 0.2	0.7 ± 0.0	0.7 ± 0.0
i/a17:0	0.3 ± 0.0	**1.0 ± 0.0**[c]	**0.5 ± 0.1**[c]	0.4 ± 0.0	0.3 ± 0.0
Sum of branched-chain (iso-, anteiso-,middle-, br-15:0a,b,c, br-17:0a,b, br18:0a,b, br-19:0a,b)	**9.4 ± 0.0**[a]	**8.7 ± 1.0**[c]	**13.5 ± 0.9**[a,c]	4.0 ± 0.2	3.4 ± 0.3
Saturated straight-chain (14:0,16:0,17:0,18:0, 20:0)	**20.4 ± 0.6**[a,b]	**57.7 ± 2.7**[b]	**50.6 ± 1.7**[a]	67.3 ± 4.9	46.5 ± 12.4
Cyclopropane fatty acids (cy-17:0ω9, cy-17:0ω7, cy-19:0ω7)	**57.1 ± 0.3**[a,b]	**17.2 ± 3.7**[b]	**5.3 ± 2.8**[a,c]	tr	tr
cy-17:0/pre	**8.2 ± 0.3**[a,b]	**3.0 ± 1.7**[b,c]	**0.6 ± 0.3**[a,c]	-	-
cy-19:0/pre	**5.5 ± 0.1**[a,b]	**2.0 ± 0.4**[b,c]	**0.2 ± 0.1**[a,c]	-	-
10Me16:0	0.9 ± 0.1	0.7 ± 0.1	1.3 ± 0.3	0.2 ± 0.1	0.2 ± 0.0
TBSA	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	n.d.	n.d.
Unsat/Sat	0.5 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	1.0 ± 0.5
Average carbon number	**292.4 ± 0.6**[a,b]	**286.3 ± 2.1**[b]	**282.6 ± 0.4**[a]	**283.3 ± 0.9**[d]	**287.4 ± 1.4**[d]

* atrazine 12.2-14.0 μg kg^{-1}, simazine 17.7- 34.8 μg kg^{-1}, BAM 5.3-32.4 μg kg^{-1}
** in surface soils 18:1μ9c excluded
*** in surface soils i-19:0 is included

4.4 MICROBIAL COMMUNITY COMPOSITIONS WITH PESTICIDE CONTAMINATION

4.4.1 Atrazine contaminated groundwater deposits

The quantities of PLFAs, GLFAs, and NLFAs, and calculated microbial cell numbers showed no significant difference between clean and atrazine-contaminated groundwater deposits, when the pipe B with nutrient flow from bank filtration of lake water was excluded (I, III). In microbial lipids, the indicators for stress and slow growth (percentages of cyclopropane FAs and cy/pre) were low. In addition, the ratios of unsat/sat and average C numbers showed high microbial activity (Table 2 a, b). As a result, the microbial communities in study environments were stabilized and microbes were adapted to atrazine (III). Only some changes in microbial lipid profiles were detected between atrazine-contaminated and clean deposits many years after application. Atrazine sale in Finland was banned in 1992. In PLFAs, atrazine effects were found in rise of the abundance of 16:1ω5c and the fall of the proportion of 18:1ω9c. The ratios of *iso*-branched acids to *anteiso*-branched FAs in PLFAs were greater in atrazine-polluted deposits than in clean ones. The increase in *iso*-branched acids in atrazine-contaminated sites could be due to the use of the isopropyl group of degraded atrazine as a priming molecule in FA biosynthesis. These results are in agreement with earlier documents showing that atrazine had effects on microbial communities in soil as well as in aquifer (Seghers et al. 2003, Iker et al. 2010). The impacts of atrazine contamination in GLFAs and NLFAs were minor compared to the PLFAs (III).

4.4.2 DDT, HCB and PeCA contaminated surface soils

These results showed that soil OM content was the key factor affecting microbial biomass in pesticide-contaminated surface soils. The long-term DDT, PeCA, and PeCA and HCB contamination had no effects on microbial biomass or the quantities of GLFAs and NLFAs (Fig.3 A, B, Fig.4 A, B). These results are consistent with earlier documents with short-range pesticide contamination (Widenfalk et al. 2008).

The amount of PLFAs in DDT, PeCA, and HCB contaminated surface soils varied between 9 to 63 μg g^{-1}. The quantities of GLFAs and NLFAs ranged from 3 to 22 μg g^{-1}, and from 13 to 66 μg g^{-1}, respectively. In uncontaminated control soils of old market gardens the quantity of PLFAs was between 5 to 45 μg g^{-1}, that of GLFAs between 6 to 25 μg g^{-1}, and that of NLFAs between 8 to 72 μg g^{-1} (IV).

Pesticide effects on microbial community composition were minor compared to those of soil OM content and vegetation, nonetheless some adaptive changes were found in the lipid profiles from soils of long-term pesticide contamination compared to those of control soils. The pesticide effects seemed to be rather similar between DDT, PeCA, and HCB contaminated soils, however, the fate of these

pesticides was different due to OM content of the soil where it was released. The results revealed that DDT and its residues persisted in higher concentrations in sandy soil with low OM content (≤5.1 % dry wt) causing only scarce impacts on FA profiles, while the occurrence was contrary in soils with higher OM content (≥7.3 % dry wt). PeCA and HCB remained in greater concentrations in soils with higher OM content (≥5.8 % dry wt), where their impacts on FA profiles were more numerous than in sandy soils (OM≤5.3 % dry wt) (IV). The bioavailablity of xenobiotics has generally been shown to reduce due to sorption to soil OM and also with time (Chaplain et al. 2011, Sudharshan et al. 2012).

The changes in FA profiles, such as the lower percentages of cyclopropane FAs, the ratios of cy/pre, and lower average C numbers, as well as the higher ratios of unsat/sat in contaminated soils, were favourable to microbes, indicating more active microbial populations in pesticide-containing soils than controls. This suggests that the toxicity of DDT, PeCA, and HCB to microbes had decreased during decades (Sudharshan et al. 2012). These results showed that the effects of long-term DDT, PeCA, and HCB pollution could be rather estimated by the modifications in microbial communities than by changes in lipid quantities (Westergaard et al. 2001, IV)

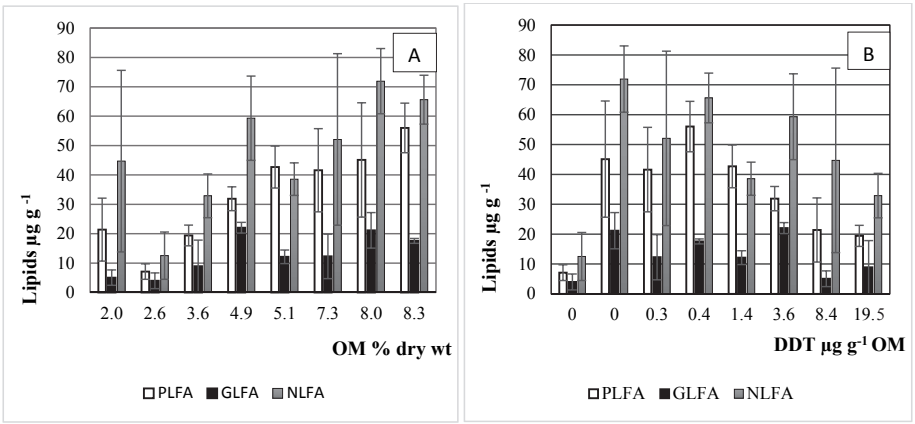

Fig. 3. PLFA, GLFA, and NLFA quantities in DDT contaminated surface soils (old market gardens) with increasing OM content (% dry wt) (A), and with increasing DDT concentration (µg g^{-1} OM) (B).

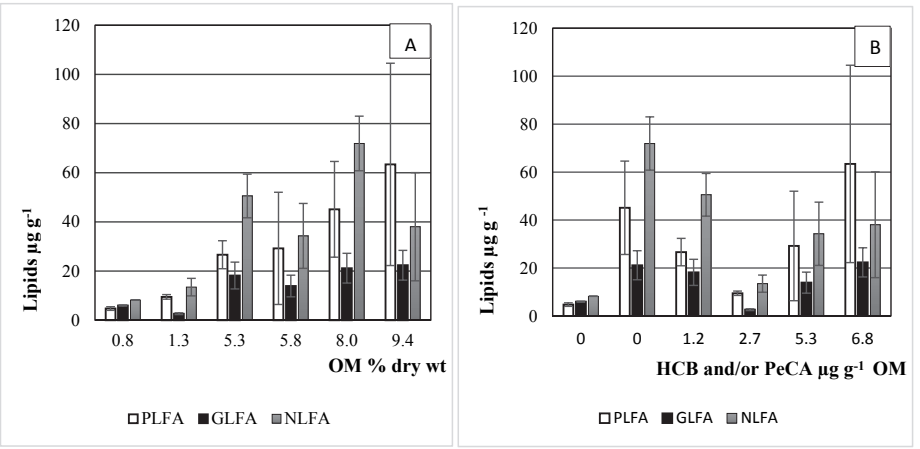

Fig.4. PLFA, GLFA, and NLFA quantities in PeCA and HCB contaminated surface soils (old market gardens) with increasing OM content (% dry wt) (A), and with increasing PeCA and HCB concentration (µg g^{-1} OM) (B).

4.5 DEPEDENCE OF PESTICIDE DEGRADATION ON MICROBIAL COMMUNITY COMPOSITION AND ENVIRONMENTAL FACTORS

The indigenous microbes from clean groundwater pipe A degraded atrazine rapidly in aerobic laboratory experiments, while in the other deposits slow anaerobic degradation occurred (I). The enhanced biodegradation in pipe A cannot be explained by higher microbial biomass or cell numbers, though high biomass has been considered to be a sign of potential biodegradation (Holden and Fierer 2005) (III). Microbial community composition differed slightly between pipe A and the other deposits. The PLFA profile in pipe A can be described with relatively high percentages of Gram-positive bacteria (*iso*- and *anteiso*-branched acids) and fungi (18:2ω6,9c) among the deposits. The quantities of GLFAs, as well as the portions of Gram-negative bacteria (monounsaturated FAs) in GLFAs, were nearly highest of the deposits. However, the lipid profiles could not provide a perfect community composition of the atrazine degraders, though it could be suggested that Gram-positive bacteria and fungi have taken part in atrazine biodegradation (III).

It has been suggested that low values of physiological indicators, such as the ratios of cyclopropyl FAs to their monoenoic precursors could be indicators for potential biodegradation in contaminated groundwater environments (Green and Scow 2000). However, the percentages of the cyclopropane FAs were extremely small in drilling sediments and groundwater deposits (Table 2 a, b, c) and could not be reliably measured, so they seemed to be inappropriate for indicators of future biodegradation in these environments (II, III). On the other hand, the average C numbers describing the FA length, and the ratio of unsaturated to saturated FAs

could be reliably calculated also in drilling sediments and groundwater deposits in PLFAs, GLFAs, and NLFAs. Consequently, the low average C numbers and high unsat/sat ratios signifying high microbial activity might be useful, when the possibilities of pesticide biodegradation are widely observed (Table 2 a, b, c, II, III, IV).

The laboratory experiment showed that microbes were successful in atrazine biodegradation with low nutrient contents (Holden and Fierer 2005, I). The higher OM content in contaminated deposits of this study could not be related to atrazine dissipation. The higher OM content in PeCA and HCB contaminated surface soils seemed to prevent the biodegradation of PCNB. On the other hand, the higher soil OM content in DDT-contaminated surface soils seemed to activate the microbial population and might possibly contribute to DDT degradation in surface soils (IV). The nearly optimal C/N-ratio and the slightest depth from the water table (0.8 m) with sufficient oxygen seemed to be associated to atrazine biodegradation. These results support the assumption that fungi might have participated in atrazine biodegradation in pipe A (III). On the other hand, the high amount of nitrogen and NH_4 in pipe B might have hindered atrazine biodegradation due to inhibiting impact of exogenous nitrogen in atrazine biodegradation (Rhine et al. 2003). In pipe C, the amounts of inorganic elements were higher than in other pipes, which might have had negative impacts on atrazine degrading microbes (III).

Pesticide biodegradation fails to occur if the necessary genes are absent. The genes for pesticide degradation activity have evolved over time with long-term pesticide contamination (Weiss and Cozzarelli 2008, Krutz et al. 2010). Soil bacteria have been reported to be able to biodegrade s-triazines, and BAM (Shapir et al. 2007, Holtze et al. 2008). However, DDT and its metabolites as well as HCB and PeCA, have remained for decades in old market garden soils in Finland, and therefore their natural biodegradation in similar environments is unlikely. Consequently, more researches are required in order to achieve bioremediation of DDT and PCNB residues in soils (IV).

5 CONCLUSIONS

Soil type and OM content seemed to predominantly influence the quantities of microbial lipids (PLFAs, GLFAs, and NLFAs) and community composition. With depth, soil OM content generally declined resulting in reduced microbial biomass and cell numbers. The variations in microbial biomass and cell numbers were the greatest on the top soil layers and less in the deeper sediments. Generally the quantity of PLFAs was higher than that of GLFAs but lower than the quantity of NLFAs. However, in groundwater deposits and deep drilling sediments phosphorous limitation or the growth of Gram-positive bacteria possibly caused higher GLFA quantities compared to PLFAs. The quantity of NLFAs seemed to depend on the OM content and amount of carbon in deposits. The stressful conditions in subsurface soils could be most reliably detected with the ratio of unsat/sat and average C numbers due to the portions of *trans*-monounsaturated and cyclopropane FAs were extremely low in drilling sediments. High portions of cyclopropane FAs and low portions of saturated straight-chain FAs were related to organic soil type, especially in GLFAs and NLFAs.

These results showed that long-term pesticide contamination in surface soils had minor impacts on microbial community composition compared to other factors such as soil type and OM content. In soils with low OM content, the microbial community structures of DDT contaminated samples resembled those of control soils more than in soils with higher OM content. The occurrence with PeCA and HCB contamination was the opposite compared to DDT. The differences between DDT, PeCA and HCB contaminated and control soils were due to microbial adaptation more than changes in microbial community composition, and were found in low proportions of cyclopropane FAs and the low ratios of cy/pre. Nonetheless, these results showed that DDT, HCB, and PeCA had an activating effect on micro-organisms regardless of soil type. The long-term impacts of atrazine were found in changes of the abundance of some monounsaturated FAs and increased proportions of *iso*-branched FAs. No effects of atrazine, DDT, PeCA, and HCB contamination could be detected in changes of microbial biomass calculated by PLFAs or the quantities of GLFAs and NLFAs.

Microbial community composition observed by PLFAs may give some clues for successful pesticide degradation; though defined bioremediation-enhancing microbial community composition could not be found by lipid analysis. Microbes in the long-lasting pollution samples seemed to be adapted to pesticides due to no stress by physiological indices in lipid profiles. Therefore, in surface soils with long-term pesticide contamination, the low ratio of cy/pre could be an appropriate indicator for potential biodegradation. Instead, in deep drilling sediments and

groundwater deposits, the ratios of cy/pre seemed to be unreliable in predicting future biodegradation. These results revealed that microbial activity detected by ratios of unsat/sat and average C numbers from lipid profiles was the highest in certain groundwater monitoring pipes and triazines and BAM-contaminated shallowest sediments, and higher with DDT, HCB, and PeCA contaminated surface soils than in clean controls. As a result, the ratios of unsat/sat and the average C numbers signifying the FA chain length might be useful to predict the possibilities of biodegradation.

6 ACKNOWLEDGEMENTS

First, I am grateful to the pre-examiners, Professor Erland Bååth and Professor Olli H. Tuovinen for their valuable comments on finishing my thesis. Secondly, I thank my supervisors Merja Kontro and Martin Romantschuk for their advice, contribution, and guidance to this work, and special thanks to Professor Rauni Strömmer, who helped me forward with her instructions. I also thank docent Hannu Fritze, who accepted the task of an opponent.

I want to express my gratitude to the great personnel of the Department of Environmental Sciences in Lahti for friendly and helpful atmosphere. I am truly grateful for the help of Vesa at crucial times with memory sticks. I want to thank all my friends, who have supported me during the years, which were divided between study and work. I am also grateful for the support of my children Soile, Essi, and Matti; you are the best. Miqets, baruch HaShem!

This study was funded by Agronomiliitto, Ekokem Corporation, EnSTe graduate school, Finnish Concordia Fund, Maj and Tor Nessling foundation, Ministry of Agriculture and Forestry of Finland, LahtiAqua Ltd, Maa- ja vesitekniikan tuki ry, and the University of Helsinki.

7 REFERENCES

Ager D., Evans S., Li H., Lilley A.K., van der Gast C.J. 2010. Anthropogenic disturbances affects the structure of bacterial communities. *Environ Microbiol* 12: 670–678.

Aislabie J., Davison A.D., Boul H.L., Franzmann P.D., Jardine D.R., Karuso P. 1999. Isolation of *Terrabacter* sp. strain DDE-1, which metabolizes 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene when induced with biphenyl. *Appl Environ Microbiol* 65: 5607–5611.

Aislabie J., Lloyd-Jones G. 1995. A review of bacterial-degradation of pesticides. *Austral J Soil Res* 33: 925–942.

Albrechtsen H.J., Winding A. 1992. Microbial biomass and activity in subsurface sediments from Vejen, Denmark. *Microb Ecol* 23: 303–317.

Allison S.D., Martiny J.B.H. 2008. Resistance, resilience, and redundancy in microbial communities. *PNAS* 12: 11512–11519.

Allison V.J., Miller R.M., Jastrow J.D., Matamala R., Zak D.R. 2005. Changes in the soil microbial community structure in a tallgrass prairie chronosequence. *Soil Sci Soc Am J* 69: 1412–1421.

Allison V.J., Yermakov Z., Miller R.M., Jastrow J.D., Matamala R. 2007. Using landscape and depth gradients to decouple the impact of correlated environmental variables on soil microbial community composition. *Soil Biol Biochem* 39: 505–516.

Atlas R.M., Bartha R. 1998. Approaches to bioremediation. In: Microbial Ecology – fundamentals and applications, 4th ed, Benjamin/Cummings Science Publishing, Menlo Park, CA.

Atlas R.M., Horowitz A., Krichevsky M., Bej A.K. 1991. Response of microbial populations to environmental disturbance. *Microb Ecol* 22: 249–256.

Bååth E. 2003. The use of the neutral lipid fatty acids to indicate the physiological conditions of soil fungi. *Microb Ecol* 45: 373–383.

Bååth E., Anderson T.-H. 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biol Biochem* 35: 955–963.

Bach E.M., Baer S.G., Meyer C.K., Six J. 2010. Soil texture affects soil microbial and structural recovery during grassland restoration. *Soil Biol Biochem* 42: 2182–2191.

Bach L.H., Frostegård Å., Ohlson M. 2008. Variation in soil microbial communities across a boreal spruce forest landscape. *Can. J. For. Res.* 38: 1504–1516.

Balkwill D.L., Leach F.R., Wilson J.T., McNabb J.F., White D.C. 1988. Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in subsurface aquifer sediments. *Microb Ecol* 16: 73–84.

Balkwill D.L., Reeves R.H., Drake G.R., Reeves J.Y., Crocker F.H., Baldwin K.M., Boone D.R. 1997. Phylogenetic characterization of bacteria in the subsurface microbial culture collection. *FEMS Microbiol Rev* 20: 201–216.

Bardgett R.D. 2005. The biology of soil. A community and ecosystem approach. Oxford University Press, Oxford.

Bardgett R.D., Kandeler E., Tscherk D., Hobbs P.J., Bezemer T.M., Jones T.H., Thompson L.J. 1999. Below-ground microbial community development in a high temperature world. *Oikos* 85: 193–203.

Bligh E.G., Dyer W.J. 1959. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37: 911–917.

Blume E., Bischoff M., Reichert J.M., Moorman T., Konopka A., Turco R.F. 2002. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. *Appl Soil Ecol* 20: 171–181.

Bossio D.A., Fleck J.A., Scow K.M., Fujii R. 2006. Alteration of soil microbial communities and water quality in restored wetlands. *Soil Biol Biochem* 38:1223–1233.

Bossio D.A., Scow K.M. 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. *Microb Ecol* 35: 265–278.

Botton S., van Heusden M., Parsons J.R., Smidt H., van Straalen N. 2006. Resilience of microbial systems towards disturbances. *Crit Rev Microbiol* 32: 101–112.

Brant J.B., Myrold D.D., Sulzman E.W. 2006. Root controls on soil microbial community structure in forest soils. *Oecologia* 148: 650–659.

Briceño G., Palma G., Durán, N. 2007. Influence of organic amendment on the biodegradation and movement of pesticides. *Crit Rev Env Sci Technol* 37: 233–271.

Brockman F.J., Murray C.J. 1997. Subsurface microbiological heterogeneity: current knowledge, descriptive approaches and applications. *FEMS Microbiol Rev* 20: 231–247.

Buckley D.H., Schmidt T.M. 2003. Diversity and dynamics of microbial communities in soils from agro-ecosystems. *Environ Microbiol* 5: 441–452.

Chaplain V., Mamy L., Vieuble´-Gonod L., Mougin C., Benoit P., Barriuso E., Nélieu S. (2011) Fate of pesticides in soils: Toward an integrated approach of influential factors, Pesticides in the Modern World – Risks and Benefits, M Stoytcheva (Ed.), InTech, pp. 535–560, ISBN: 978-953-307-458-0.

Cronan J.E. Jr. 2002. Phospholipid modifications in bacteria. *Curr Opin Microbiol* 5: 202–205.

Das A.C., Mukherjee D. 2000. Soil application of insecticides influences microorganisms and plant nutrients. *Appl Soil Ecol* 14: 55–62.

de Lipthay J.R., Johnsen K., Albrechtsen H.J., Rosenberg P., Aamand J. 2004. Bacterial diversity and community structure of a sub-surface aquifer exposed to realistic low herbicide concentrations. *FEMS Microbiol Ecol* 49: 59–69.

Downing H.F., Delorenzo M.E., Fulton M.H., Scott G.I., Madden C.J., Kucklick J.R. 2004. Effects of the agricultural pesticides atrazine, chlorothalonil, and endosulfan on south Florida microbial assemblages. *Ecotoxicology* 13: 245–260.

Drenovsky RE, Steenwerth KL, Jackson LE, Scow KM (2010) Land use and climatic factors structure regional patterns in soil microbial communities. *Global Ecol Biogeogr* 19: 27–39.

Eganhouse R.P., Pontolillo J. 2008. DDE in sediments of the Palos Verdes shelf, California: In situ transformation rates and geochemical fate. *Environ Sci Technol* 42: 6392–6398.

Federle T.W., Dobbins D.C., Thornton-Manning J.R., Jones D.D. 1986. Microbial biomass, activity, and community structure in subsurface soils. *Ground Water* 24: 365–374.

Feng X., Nielsen L., Simpson M.J. 2007. Responses of soil organic matter and microorganisms to freeze-thaw cycles. *Soil Biol Biochem* 39: 2027–2037.

Fierer N., Jackson R.B. 2006. The diversity and biogeography of soil bacterial communities. *PNAS* 17: 626–631.

Fierer N., Schimel J.P., Holden P.C. 2003. Variations in microbial community composition through two soil depth profiles. *Soil Biol Biochem* 35: 167–176.

Fierer N., Strickland M.S., Liptzin D., Bradford M.A., Cleveland C.C. 2009. Global patterns in belowground communities. *Ecol Lett* 12: 1238–1249.

Fischer J., Schauer F., Heipieper H.J. 2010. The *trans/cis* ratio of unsaturated fatty acids is not applicable as biomarker for environmental stress in case of long-term contaminated habitats. *Appl Microbiol Biotechnol* 87: 365–371.

Frostegård Å., Bååth E. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol Fertil Soils* 22: 59–65.

Frostegård Å., Bååth E., Tunlid A. 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biol Biochem* 25: 723–730.

Frostegård Å., Tunlid A., Bååth E. 2010. Use and misuse of PLFA measurements in soils. *Soil Biol Biochem,* doi:10.1016/j.soilbio.2010.11021.

García C., Roldan A., Hernandez T. 1997. Changes in microbial activity after abandonment of cultivation in a semiarid Mediterranean environment. *J Environ Qual* 26: 285–292.

Gaw S. K., Wilkins A. L., Kim N. D., Palmer G.T., Robinson P. 2005. Trace element and ΣDDT concentrations in horticultural soils from Tasman, Waikato and Auckland regions of New Zealand. *Sci Total Environ* 355: 31–47.

Goldscheider N., Hunkeler D., Rossi P. 2006. Microbial biocenoses in pristine aquifers and an assessment of investigative methods. *Hydrogeol J* 14: 926–941.

Grayston S.J., Griffith G.S., Mawdsley J.L., Campbell C.D., Bardgett R.D. 2001. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. *Soil Biol Biochem* 33: 533–551.

Green C.T., Scow K.M. (.2000. Analysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers. *Hydrol J* 8: 126–141.

Griebler C., Lueders T. 2009. Microbial biodiversity in groundwater ecosystems. *Freshwater Biol* 54: 649–677.

Griffiths B.S., Bonkowski M., Roy J., Ritz K. 2001. Functional stability, substrate utilization and biological indicators of soils following environmental impacts. *Appl Soil Ecol* 16: 49–61.

Griffiths R.I., Whiteley A.S., O´Donnell A.G., Bailey M.J. 2003. Influence of depth and sampling time on bacterial community structure in an upland grassland soil. *FEMS Microbiol Ecol* 43: 35–43.

Haack S.K., Fogarty L.R., West T.G., Alm E.W., McGuire J.T., Long D.T., Hyndman D.W., Forney L.J. 2004. Spatial and temporal changes in microbial community structure associated with recharge-influenced chemical gradients in a contaminated aquifer. *Environ Microbiol* 6: 438–448.

Habekost M., Eisenhauer N., Scheu S., Steinbeiss S., Weigelt A., Gleixner G. 2008. Seasonal changes in the soil microbial community in a grassland plant diversity gradient four years after establishment. *Soil Biol Biochem* 40: 2588–2595.

Haney R.L., Senseman S.A., Hons F.M., Zuberer D.A. 2000. Effect of glyphosate on soil microbial activity and biomass. *Weed Sci* 48: 89–93.

Hedrick D.B., Peacock A., Stephen J.R., Macnaughton S.J., Brüggemann J., White D.C. 2000. Measuring soil microbial community diversity using polar lipid fatty acid and denaturing gradient gel elecrtrophoresis data. *J Microbiol Meth* 41: 235–248.

Heipieper H.J., Meinhardt F., Segura A. 2003. The *cis-trans* isomerase of unsaturated fatty acids in *Pseudomonas* and *Vibrio*: biochemistry, molecular biology and physiological function of a unique stress adaptive mechanism. *FEMS Microbiol Lett* 229: 1–7.

Högberg M.N., Högberg .P, Myrold D.D. 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? *Oecologia* 150: 590–601.

Holden P.A., Fierer N. 2005. Microbial processes in the vadose zone. *Vadose Zone J* 4: 1–21.

Holtze M.S., Sørensen S.R., Sørensen J., Aamand J. 2008. Microbial degradation of the benzonitrile herbicides dichlobenil, bromoxynil and ioxynil in soil and subsurface environments – Insights into degradation pathways, persistent metabolites and involved degrader organisms. *Environ Pollut* 154: 155–168.

Huang G., Li Q., Zhang X. 2003. Adsorption and desorption of atrazine by three soils. *Bull Environ Contam Toxicol* 71: 655–661.

Hughes J.B., Hellmann J.J., Ricketts T.H., Bohannan B.J. 2001. Counting the uncountable: statistical approaches to estimating microbial diversity. *Appl Environ Microbiol* 67: 4399–4406.

Humbert J.F., Dorigo U. 2005. Biodiversity and aquatic ecosystem functioning: a mini review. *Aq Ecosystem Health Manag* 8: 367–374.

Humphries J.A., Ashe A.M.H., Smiley J.A., Johnston C.G. 2005. Microbial community structure and trichloroetylene degradation in groundwater. *Can J Microbiol* 51: 433–439.

Ibekwe A.M., Kennedy A.C. 1998. Phospholipid fatty acid profiles and carbon utilization patterns for analysis of microbial community structure under field and greenhouse conditions. *FEMS Microbiol Ecol* 26: 151–163.

Iker B.C., Kambesis P., Oehrle S.A., Groves C., Barton H.A. 2010. Microbial atrazine breakdown in a karst groundwater system and its effect on ecosystem energetics. *J Environ Qual* 39:509–518.

Jaakkonen S., Sorvari J. 2006. Environmental effects and risk assessment of pesticide use in forest tree nurseries. Uudenmaan ympäristökeskus (In Finnish). ISBN: 952-11-2186-6 (PDF).

Jablonowski N.D., Modler J., Schaeffer A., Burauel P. 2008. Bioaccessibility of environmentally aged 14C atrazine residues in an agriculturally used soil and its particle-size aggregates. *Environ Sci Technol* 42: 5904–5910.

Jacobsen C. S., Hjelmsø M. H. 2014. Agricultural soils, pesticides and microbial diversity. *Curr Opin Biotech* 27: 15–20.

Johnsen K.J., Jacobsen C.S., Torsvik V., Sørensen J. 2001. Pesticide effects on bacterial diversity in agricultural soils – a review. *Biol Fertil Soils* 33: 443–453.

Johnson A., Llewellyn N., Smith J., van der Gast C., Lilley A., Singer A., Thompson I. 2004. The role of microbial community composition and groundwater chemistry in determing isopruton degradation potential in UK aquifers. *FEMS Microbiol Ecol* 49: 71–82.

Jolly I.D., McEwan K.L., Holland K.L. 2008. A review of groundwater-surface water interactions in arid/semi-arid wetlands and the consequences of salinity for wetland ecology. *Ecohydrol* 1: 43–58.

Jörgensen R.G., Emmerling C. 2006. Methods for evaluating human impact on soil microorganisms based on their activity, biomass, and diversity in agricultural soils. *J Plant Nutr Soil Sci* 169: 295–309.

Kalia A., Gosal S. K. 2011. Effects of pesticide application on soil microorganisms. *Arch Agron Soil Sci* 57: 569–596.

Kantachote D., Naidu R., Singleton I., McClure N., Harch B.D. 2001. Resistance of microbial populations in DDT-contaminated soils. *Appl Soil Biol* 16: 85–90.

Kantachote D., Singleton I., McClure N., Naidu R., Megharaj M., Harch B.D. 2003. DDT resistance and transformation by different microbial strains isolated from DDT-contaminated soils and compost materials. *Comp Sci Util* 11: 300–310.

Katsoyiannis A., Samara C. 2004. Persistent organic pollutants (POPs) in the sewage treatment plant of Thessaloniki, northern Greece: occurrence and removal. *Water Res* 38: 2685–2698.

Keinänen, M.M., Korhonen L.K., Martikainen P.J., Vartiainen T., Miettinen I.T., Lehtola M.J., Nenonen K., Pajunen H., Kontro M.H. 2003. Gas chromatographic-mass spectrometric detection of 2- and 3-hydroxy fatty acids as methyl esters from soil, sediment and biofilm. *J Chromatogr B* 738: 443–451.

Keith-Roach M.J., Bryan N.D., Bardgett R.D., Livens F.R. 2002. Seasonal changes in the microbial community of a salt marsh, measured by phospholipid fatty acid analysis. *Biogeochemistry* 60: 77–96.

Kieft T.L., Rinkelberg D.B., White D.C. 1994. Changes in the ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. *Appl Environ Microbiol* 60: 3292–3299.

Klamer M., Bååth E. 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acids analysis. *FEMS Microbiol Ecol* 27: 9–20.

Kontro M., Korhonen L., Vartiainen T., Pellikka P., Martikainen P.J. 2006. Selected ion monitoring in quantitative gas-liquid chromatographic-mass spectrometric detection of fatty acid methyl esters from environmental samples. *J Chromatogr B* 831: 281–287.

Kramer C., Gleixner G. 2008. Soil organic matter in soil depth profiles: distinct carbon preferences of microbial groups during carbon transformation. *Soil Biol Biochem* 40: 425–433.

Krutz L.J., Shaner D.L., Weaver M.A., Webb R.M.T., Zablotowicz R.M., Reddy K.N., Huang Y., Thomson S. 2010. Agronomic and environmental implications of enhanced s-triazine degradation. *Pest Manag Sci* 66: 461–481.

Lauber C.L., Strickland M.S., Bradford M.A., Fierer N. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol Biochem* 40: 2407–2415.

Lechevalier H., Lechevalier M.P. 1977. Chemotaxonomic use of lipids – an overview. In: Microbial lipids, Ratledge C, Wilkinson SG (eds), Vol 1, Academic Press, London, 869–902.

Lõhmus K., Truu M., Truu J., Ostonen I., Kaar E., Vares A., Uri V., Alama S., Kanal A. 2006. Functional diversity of culturable bacterial communities in the rhizosphere in relation to fine-root and soil parameters in alder stands on forest, abandoned agricultural, and oil-shale mining areas. *Plant Soil* 283: 1–10.

Megharaj M., Kantachote D., Singleton I., Naidu R. 2000. Effects of long-term contamination of DDT on soil microflora with special reference to soil algae and algal transformation of DDT. *Environ Pollut* 109: 35–42.

Meijer S.N., Halsall C.J., Harner T., Peters J.N., Ockenden W.A., Johnston A.E., Jones K.C. 2001. Organochlorine pesticide residues in archived UK soil. *Environ Sci Technol* 35: 1989–1995.

Miettinen I.T., Vartiainen T., Martikainen P.J. 1996. Contamination of drinking water. *Nature* 381: 654–655.

Mrozik A., Łabużek S., Pietrowska-Seget Z. 2005. Changes in fatty acid composition in *Pseudomonas putida* and *Pseudomonas stutzeri* during naphthalene degradation. *Microbiol Research* 160: 149–157.

Muir D., Sverko E. 2006. Analytical methods for PCBs and organochlorine pesticides in environmental monitoring and surveillance: a critical appraisal. *Anal Bioanal Chem* 386: 769–789.

Muñoz-Rojas J., Bernal P., Duque E., Godoy P., Segura A., Ramos J.L. 2006. Involvement of cyclopropane fatty acids in the response of *Pseudomonas putida* KT2440 to freeze-drying. *Appl Environ Microbiol* 72: 472–477.

Muyzer G., Stams A.J.M. 2008. The ecology and biotechnology of sulfate-reducing bacteria. *Nature Rev Microbiol* 6: 441–454.

Nannipieri P., Ascher J., Ceccherini M.T., Landi L., Pietramellara G., Renella G. 2003. Microbial diversity and soil functions. *Eur J Soil Sci* 54: 655–670.

Närhi, I. 2008. Torjunta-aineiden esiintyminen maaperässä ja pohjavedessä lopettaneilla taimi- ja kauppapuutarhoilla. Länsi-Suomen ympäristökeskus (In Finnish). ISBN:978-952-11-3291-9.

Nielsen L.E., Kadavy D.R., Rajagopal S., Drijber R., Nickerson K.W. 2005. Survey of extreme solvent tolerance in Gram-positive cocci: membrane fatty acid changes in *Staphylococcus haemolyticus* grown in toluene. *Appl Environ Microbiol* 71: 5171–5176.

Odum E.P. 1985. Trends expected in stressed ecosystems. *BioScience* 35: 419–422.

Okutman T.D., Pavlostathis S.G. 2007. Temperature and pH effect on the microbial reductive transformation of pentachloronitrobenzene. *J Agric Food Chem* 55: 5390–5398.

Øvreås L. 2000. Population and community level approaches for analyzing microbial diversity in natural environments. *Ecol Lett* 3: 236–251.

Pedersen K., Arlinger J., Eriksson S., Hallbeck A., Hallbeck L., Johansson J. 2008. Numbers, biomass, and cultivable diversity of microbial populations relate to depth and borehole-specific conditions in groundwater from depths of 4–450 m in Olkiluoto, Finland. *ISME J* 2: 760–775.

Pennanen T., Frostegård Å., Fritze H., Bååth E. 1996. Phospholipid fatty acid composition and heavy metal tolerance of soil microbial communities along two heavy metal-polluted gradients in coniferous forests. *Appl Environ Microbiol* 62: 420–428.

Pennanen T., Strömmer R., Markkola A., Fritze H. 2001. Microbial and plant community structure across the primary succession gradient. *Scand J For Res* 16: 37–43.

Peterson G., Allen C.R., Holling C.S. 1998. Ecological resilience, biodiversity and scale. *Ecosystems* 1: 6–18.

Pfiffner S.M., Cantu J.M., Smithgall A., Peacock A.D., White D.C., Moser D.P., Onstott T.C., van Heerden E. 2006. Deep subsurface microbial biomass and community structure in Witwaterstrand basin mines. *Geomicrobiol J* 23: 431–442.

Pfiffner S.M., Palumbo A.V., Gibson T., Ringelberg D.B., McCarthy J.F. 1997. Relating ground water and sediment chemistry to microbial characterization at a BTEX-contaminated site. *Appl Biochem Biotech* 63–65: 775–788.

Pombo S.A., Pelz O., Schroth M.H., Zeyer J. 2002. Field-scale 13C-labeling of phospholipid fatty acids (PLFA) and dissolved inorganic carbon: tracing acetate assimilation and mineralization in a petroleum hydrocarbon-contaminated aquifer. *FEMS Microbiol Ecol* 41: 259–267.

Qui X., Davis J.W. 2004. Environmental bioavailability of hydrophobic organochlorines in sediments – a review. *Remediation J Spring* 14: 55–84.

Ramos J.L., Duque E., Rodríquez-Herva J.J., Godoy P., Haïdour A. 1997. Mechanism for solvent tolerance in bacteria. *J Biol Chem* 272: 3887–3890.

Ranjard L., Richaume A. 2001. Quantitative and qualitative microscale distribution of bacteria in soil. *Res Microbiol* 152: 707–716.

Rapport D.J., Whitford W.G. 1999. How ecosystems respond to stress. *BioScience* 49: 193–202.

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

Rhine E.D., Fuhrmann J.J., Radosevich M. (2003) Microbial community responses to atrazine exposure and nutrient availability: linking degradation capacity to community structure. *Microb Ecol* 46: 145–160.

Ringelberg D., Richmond M., Foley K., Reynolds C. 2008. Utility of lipid biomarkers in support of bioremediation efforts at army sites. *J Microbiol Meth* 74: 17–25.

Ros M., Goberna M., Moreno J.L., Hernandez T., García C., Insam H., Pascual J.A. 2006. Molecular and physiological bacterial diversity of a semi-arid soil contaminated with different levels of formulated atrazine. *Appl Soil Ecol* 34: 93–102.

Ruess L., Chamberlain P.M. 2010. The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. *Soil Biol Biochem* 42: 1898–1910.

Šajbidor J. 1997. Effect of some environmental factors on the content and composition of microbial membrane lipids. *Crit Rev Biotechnol* 17: 87–103.

Schmitt A., Glaser B., Borken W., Matzner E. 2008. Repeated freeze-thaw cycles changed organic matter quality in atemperate forest soil. *J Plant Nutr Soil Sci* 171: 707–718.

Seghers D., Siciliano S.D., Topp E.M., Verstraete W. 2005. Combined effect of fertilizer and herbicide applications on the abundance, community structure and performance of the soil methanotrophic community. *Soil Biol Biochem* 37: 187–193.

Seghers D., Verthé K., Rheul D., Bulcke R., Siciliano S..D, Verstraete W., Topp E.M. 2003. Effect of long-term herbicide applications on the bacterial community structure and function in an agricultural soil. *FEMS Microbiol Ecol* 46: 139–146.

Shapir N., Mongodin E.F., Sadowsky M.J., Daugherty S.C., Nelson K.E., Wackett L.P. 2007. Evolution of catabolic pathways: genomic insights into microbial s-triazine metabolism. *J Bact* 189: 674–682.

Sikkema J., de Bont J.A.M., Poolman B. 1995. Mechanism of membrane toxicity of hydrocarbons. *Microbiol Rev* 59: 201–222.

Stevens J.L., Northcott G.L., Stern G.A., Tomy G.T., Jones K.C. 2003. PAHs, PCBs, PCNs, organochlorine pesticides, synthetic musk, and polychlorinated *n*-alkanes in U.K. sewage sludge: survey results and implications. *Environ Sci Technol* 37: 462–467.

Sudharshan S., Naidu R., Mallavarapu M., Bolan N. 2012. DDT remediation in contaminated soils: a review of recent studies. *Biodegradation* 23: 851–863.

Suutari M., Laakso S. 1994. Microbial fatty acids and thermal adaptation. *Crit Rev Microbiol* 20: 285–328.

Sylvia D., Furhmann J., Hartel P., Zuberer D. 2005. Principles and applications of soil microbiology. Pearson Prentice Hall, Pearson Education Inc, Upper Saddle River, NJ.

Taylor J.P., Wilson B., Mills M.S., Burns R.G. 2002. Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. *Soil Biol Biochem* 34: 387–401.

Thompson I.P., Bailey M.J., Ellis R.J., Maquire N., Meharg A.A. 1999. Response of soil microbial communities to single and multiple doses of an organic pollutant. *Soil Biol Biochem* 31: 95–105.

Torsvik V., Øvreås L. 2002. Microbial diversity and function in soil: from genes to ecosystem. *Curr Opin Microbiol* 5: 240–245.

Torsvik V., Øvreås L. 2007. Microbial Phylogeny and Diversity in Soil. In: Modern soil microbiology, Van Elsas JD, Jansson JK, Trevors JT (eds), 2nd ed. CRC Press Boca Raton, 23–54.

Tunlid A., Ringelberg D., Phelps T.J., Low C., White D.C. 1989. Measurement of phospholipid fatty acids at picomolar concentrations in biofilms and deep subsurface sediments using gas chromatography and chemical ionization mass spectrometry. *J Microbiol Methods* 10: 139–153.

Van der Meer J.R., Werlen C., Nishino S.F., Spain J.C. 1998. Evolution of a pathway for chlorobenzene metabolism leads to natural attenuation in contaminated groundwater. *Appl Environ Microbiol* 64: 4185–4196.

Van Elsas J.D., Torsvik V., Hartmann A., Øvreås L., Jansson J.K. 2007. The bacteria and archaea in soil. In: Modern soil microbiology, Van Elsas, J D, Jansson, JK, Trevors J T (eds), 2nd ed. CRC Press Boca Raton , 23–54.

Wang M.C., Liu Y.H., Wang Q., Gong M., Hua X.M., Pang Y.J., Hu S., Yang Y.H. 2008. Impacts of methamidophos on the biochemical, catabolic, and genetic characteristics of soil microbial communities. *Soil Biol Biochem* 40: 778–788.

Weber F.J., de Bont J.A.M. 1996. Adaptation mechanisms of microorganisms to the toxic effects of organic solvents on membranes. *Biochim Biophys Acta* 1286: 225–245.

Weiss J.V., Cozzarelli I.M. 2008. Biodegradation in contaminated aquifers: incorporating microbial/molecular methods. *Ground Water* 46: 305–322.

Welp G., Brümmer G.W. 1999. Effects of organic pollutants on soil microbial activity: the influence of sorption, solubility, and speciation. *Ecotoxicol Eniron Safety* 43: 83–90.

Westergaard K., Mùller A.K., Christensen S., Bloem J., Sørensen S.J. 2001. Effects of tylosin as a disturbance on the soil microbial community. *Soil Biol Biochem* 33: 2061–2071.

White D.C., Davis W.M., Nickels J.S., King J.D., Bobbie R.J. 1979. Determination of the sedimentary microbial biomass by extractible lipid phosphate. *Oecologia* 40: 51–62.

White D.C., Flemming C.A., Leung K.T., Macnaughton S.J. 1998. In situ microbial ecology for quantitative appraisal, monitoring, and risk assessment of pollution remediation in soils, subsurface, the rhizosphere and in biofilms. *J Microbiol Methods* 32: 93–105.

White D.C., Stair J.O., Ringelberg D.B. 1996. Quantitative comparison of *in situ* microbial biodiversity by signature biomarker analysis. *J Ind Microbiol* 17: 185–196.

Wick L.Y., Pasche N., Bernasconi S.M., Pelz O., Harms H. 2003. Characterization of multiple-substrate utilization by anthracene-degrading *Mycobacterium frederiksbergense* LB501T. *Appl Environ Microbiol* 69: 6133–6142.

Widenfalk A., Bertilsson S., Sundh I., Goedkoop W. 2008. Effects of pesticides on community composition and activity of sediment microbes – responses at various levels of microbial community organization. *Environ Poll* 152: 576–584.

Zak D.R., Holmes W.E., White D.C., Peacock A.D., Tilman D. 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? *Ecology* 84: 2042–2050.

Zelles L. 1999. Fatty acid patterns of phospholipid and lipopolysaccharides in the characterisation of microbial communities in soil: review. *Biol Fertil Soils* 29: 111–129.

Zelles L., Rackwitz R., Bai Q.Y., Beck T., Beese F. 1995. Discrimination of microbial diversity by fatty acid profiles of phospholipids and lipopolysaccharides in differently cultivated soils. *Plant Soil* 170: 115–122.

Zhang C., Liu X., Dong F., Xu J., Zheng Y., Li J. 2010. Soil microbial communities response to herbicide 2,4-dichlorophenoxyacetic acid butyl ester. *Eur J Soil Biol* 46: 175–180.

Zhou J., Beicheng X., Treves D.S., Wu L.Y., Marsh T.L., O´Neill R.V., Palumbo A.V., Tiedje, J.M. 2002. Spatial and resource factors influencing high microbial diversity in soil. *Appl Environ Microbiol* 68: 326–334.