Microbial ecology in atrazine and terbutryn dissipation in surface soils and subsurface sediments

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ABSTRACT

The worldwide use of triazines as pesticides has resulted in their widespread occurrence in groundwater. Within the northern boreal region in Southern Finland, 30% of groundwater sampling points contained pesticides, and acceptable drinking water limits were exceeded in 11%. Atrazine and its degradation products were among the most common pesticides observed. Chemical pesticide degradation in soil is slower than microbial degradation. Biodegradation often decreases with increasing depth. Nutritional factors and soil physicochemical properties affect microbial pesticide degradation. However, their effects on separate microorganisms, and the genetic basis of these responses, are not well documented. The aim of this thesis was to isolate and characterize atrazine-degrading microorganisms, and to find appropriate microbial, physicochemical, and nutritional demands for pesticide degradation. Microbial community composition was studied in farmland, forested farmland, and primary forest soils by pyrosequencing and in gardens, groundwater deposits, and vadose zone sediments by cultivation on mineral medium with atrazine or terbutryn as the nitrogen source. Atrazine dissipation efficiency was additionally compared under stagnant and circulating water conditions. The dominant phyla that increased in atrazine-treated farmland, gardens, deposits, and sediments were Proteobacteria and Actinobacteria. The overlap in genera was less than in phyla, while the isolated *Pseudomonas* strains only slightly overlapped between isolates from surface soils and subsurface deposits and sediments. Atrazine dissipation was better in circulating than in stagnant water, and aerobic microbes from genera known to have atrazine degradation genes, all from phyla Proteobacteria and Actinobacteria, were simultaneously enriched,. Based on the results, the application of microbial remediation of atrazine and terbutryn requires special attention to soil physicochemical properties and selection of proper microbial strains.

LIST OF ORGINAL PAPERS

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

I. **Liu X**, Hui CL, Bi LZ, Romantschuk M, Kontro M, Strömmer R, Hui N (2016) Bacterial community structure in atrazine treated reforested farmland in Wuying China. Applied Soil Ecology 98:36-46

II. **Liu X**, Kontro M (2016). The physicochemical conditions of isolation source determine the occurrence of *Pseudomonas fluorescens* group species Annals of Microbiology 65 (4): 2363-2377. doi 10.1007/s13213-015-1078-1

III. **Liu X** and Kontro M (2015) Degradation of high atrazine load in groundwater sediments was enhanced in circulating water more than in stagnant slurry, and nitrogen released enhanced growth of bacteria related to remediation. (Manuscript)

THE AUTHOR'S CONTRIBUTION

I. First author. XL planned the field sampling with NH. XL performed the laboratory and data analyses and wrote a significant part of the paper together with corresponding author NH under the supervision of MR and MK.

II. Corresponding author. XL established the laboratory trial under the supervision of MK. XL performed the laboratory and data analyses and wrote the paper with MK.

III. Corresponding author. XL planned the laboratory trial under the supervision of MK and conducted the laboratory and data analyses. XL performed the statistical analyses and wrote the paper under the supervision of MK.

ABBREVIATIONS

ANOVA Analysis of variance

Atr Atrazine

atzA Atrazine chlorohydrolase

atzB Hydroxyatrazine ethylaminohydrolase

atzC *N*-isopropylammelide isopropylaminohydrolase

atzD Cyanuric acid hydrolase

atzE Biuret hydrolase

atzF Allophanate hydrolase

DEA Desethylatrazine

DEDIA Desethyldeisopropylatrazine

DIA Deisopropylatrazine

DGGE Denaturing Gradient Gel Electrophoresis

DNA Deoxyribonucleic acid

DOM Dissolved organic matter

EMBL European Molecular Biology Laboratory

Fst Fixation Index-Statistics (population genetics)

HPLC High Pressure Liquid Chromatography

Mesocosm An experimental tool that brings a small part of the natural environment under controlled conditions

OM Organic matter

OTU Operational taxonomic unit

PCA Principal Component Analysis

PCR	Polymerase Chain Reaction
PLFA	Phospholipid fatty acid
RDP	Ribosomal Database Project
SC	Sequencing and cloning technology
Ter	Terbutryn
trzD	Cyanuric acid amidohydrolase
trzF	Allophanate hydrolase
trzN	*S*-triazine hydrolase
US EPA	United States Environmental Protection Agency
16S rRNA	Small subunit of the ribosomal ribonucleic acid

1 INTRODUCTION

The practice of agriculture first began approximately 11 000 years ago, which was credited as a turning point in human history. Crop cultivation allowed for a more settled population and became an important part of human life (Kislev et al. 2004). Nowadays, agricultural production is one of the largest and most important economic activities in the world, and especially in agricultural countries it provides a significant impact on gross domestic product (GDP) growth. It is clear that farmed crops would suffer from insects, diseases, and weeds causing a large loss in yield (Fahad et al. 2015), e.g. weeds cause an estimated 13% loss on the global crop yield (Pimentel 2009). Pesticides were invented and applied to protect crops from certain bacteria, insects, and other potentially damaging organisms. Pesticides include insecticides, molluscicides, nematocides, fungicides, and herbicides. The history of pesticides can be tracked back 3000 years, when the Chinese used sulfur to control bacteria and fungus (National Academy of Science, 1969). The first synthetic organic pesticide was invented in 1939 (Swiss patent 226180, 1943). Since then most pesticides have been synthesized by humans and their worldwide application has grown significantly, with approximately 2.5 million tons of pesticides now used annually (Alavanja 2009).

Not only do pesticides control agricultural pests and prevent crop losses, they also control human/livestock disease vectors and harmful organisms to human activities and structures (Cooper and Dobson 2007). For example, herbicides can control weeds in agricultural fields and in urban or rural areas where they can ensure that roads, railways, and waterways are kept free of vegetation. Though pesticides have contributed to global agricultural growth and human well-being, they are often harmful to more than just their target species. The major portion of applied pesticides does not quickly degrade at the application point, and end up entering the larger environment, potentially contaminating it. Currently great interest exists in bioremediation, particularly concerning pesticides with high toxicity or persistence in the environment.

1.1 Atrazine

Atrazine (2-chloro-4-ethylamino-6-isopropylamine-s-triazine, $C_8H_{14}ClN_5$, Fig. 1) belongs to the group of s-triazine herbicides. They inhibit photosynthesis by interrupting the electron transport system. Atrazine was invented in the 1950s and quickly became the most popular of the triazines, due to its effectiveness against a wide spectrum of weeds. It has since become one of the most widely researched herbicides to date. Atrazine is moderately hydrophilic with water solubility of 33 mg l^{-1} at 22 ℃ (Tomlin, 2000). The ideal pesticide should be toxic only to the target weeds, and easily biodegradable. It should not enter the surrounding areas or leach into groundwater. Unfortunately, this is rarely the case. Atrazine is degraded in the soil relatively rapidly with half-lives of 32 to 128 days (Krutz et al. 2008). Its half-life is considerably longer in subsurface environments. In groundwater monitoring pipes and well deposits, atrazine degradation under aerobic conditions varied from rapid (half-life 38 days) to no degradation.

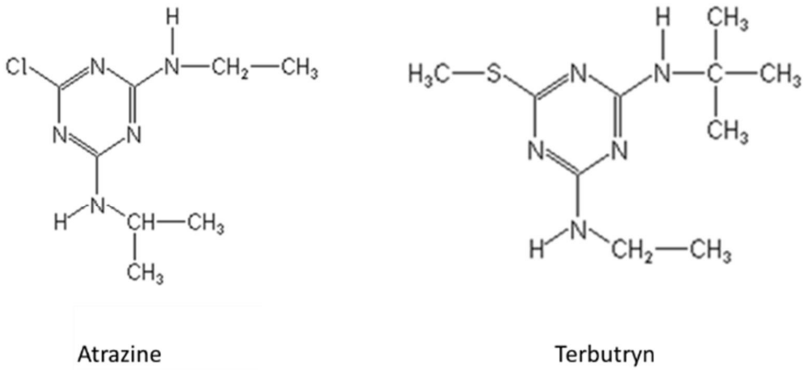

Fig. 1 Chemical structures of atrazine and terbutryn

Anaerobically, atrazine half-lives were 430 to 829 days (Talja et al. 2008). Atrazine is identified as an endocrine-disrupting agent, which is slightly to moderately toxic to humans and animals. The European Union banned the sale of atrazine in 1992, due to its persistence in groundwater and potential threat to drinking water resources.

Atrazine and its degradation products e.g. desethylatrazine (DEA), deisopropylatrazine (DIA) and desethyldeisopropylatrazine (DEDIA)were among the most commonly observed compounds in groundwater in Finland (Vuorimaa et al. 2007), due to urban activities, not farming. The slow leaching of pesticides and degradation products from the clay soil into the groundwater was suggested to be related to the long duration problem of pesticides in groundwater reservoirs in Finland (Mattsson et al. 2015). Although the use of atrazine is banned in Finland, it remains the most widely and heavily used herbicide in other nations outside the European Union such as Brazil, China, India, and Russia. Over 3.4 x 10^4 t of atrazine is applied each year in the USA (Sadler et al. 2014). The expected amount of yearly atrazine usage in China in 2020 is 10820t (Zhang et al. 2014).

1.2 Terbutryn

Terbutryn (2-t-butylamino-4-ethylamino-6-methylthio-s-triazine, Fig.1) was invented in the 1970s. It inhibits photosynthesis by interrupting the electron transport system. In addition to preventing wild grass growth, terbutryn is also applied as a biocide in paints, due to its ability to prevent the growth of algae on building surfaces (Burkhardt et al. 2012). Terbutryn is moderately hydrophilic, with a water solubility of 22 mg l^{-1} at 22℃ and its soil half-life is 14–50 days (Tomlin, 2000). Many commercial products containing terbutryn are sold in more than 25 countries, including Mexico, the Ivory Coast, Spain, Guatemala, and the United Kingdom (LeBaron et al., 2008). Terbutryn can be transported to surface water and subsoil by runoff precipitation, and leach through subsurface soil to the groundwater. The Environmental Protection Agency of the United States has classified terbutryn as

belonging to Toxicity Class III – slightly toxic and a possible human carcinogen. Terbutryn has negative effects on aquatic non-target organisms, e.g. common carps are affected by long-term exposure to environmental concentrations of terbutryn at levels of 0.2–2.0 μg l^{-1} (Velisek et al. 2012).

As many triazine herbicides, terbutryn has a tendency to move from the soil to surface or subsurface waterbodies through water runoffs and leaching (Fenoll et al. 2014). The European Union banned terbutryn in 2003, due to it persistence in groundwater and potential threat to drinking water resources. However, a sale ban does not necessarily lead to the immediate disappearance of the banned substance from the environment. Years after the end of terbutryn application, its concentration exceeded the limit of EU legislation without a visible trend of decreasing concentration in a river in Germany (Quednow and Puttmann 2007), and in wastewater treatment plants in Spain (Matamoros and Salvadó 2013). The major degradation product of terbutryn is hydroxy-terbutryn (2-hydroxy-4-tert-butylamino-6-ethylamino-s-triazine), and to a minor extent terbutryn sulfoxide (2-methylsulfinyl-4-tert-butylamino-6-ethylamino-s-triazine) and N-deethylated terbutryn (2-methylthio-4-tert-butylamino-6-amino-s-triazine). Although the possibility of terbutryn biodegradation was discussed a long time ago (Muir and Yarechewski 1982), much less information exists on the fate and degradation of terbutryn in soil and water. Muir and Yarechewski (1982) reported 180-day degradation half-lives for terbutryn in river sediments under laboratory conditions. Aerobic and anaerobic terbutryn half-lives were 193–644 and 266–400 days in groundwater monitoring pipes and well deposits, respectively (Talja et al. 2008).

1.3 Atrazine and terbutryn degradation

According to Finnish authorities, based on European Union legislation, the permitted value of a single pesticide in groundwater is 0.10 μg l^{-1}, and the sum of different pesticides should not exceed 0.50 μg l^{-1} (European Union, 1998). Recently 164 groundwater samples were investigated from 23 European countries; 20% of the samples contained at least one pesticide contaminant that exceeded the EU limit, and 10% exceeded the limit of multiple pesticides. The frequency of detected atrazine was 56% and its maximum concentration was 253 ng l^{-1}. Atrazine degradation product desethylatrazine was detected at a frequency of 52%, with a maximum concentration of 487 ng l^{-1}, and at six sites its concentration exceeded the European groundwater quality standard of 0.10 μg l^{-1} for one pesticide or degradation product. The results showed that the occurrence of atrazine and its degradation products in groundwater is not negligible in Europe (Loos et al. 2010). Several physicochemical technologies are available for the cleanup of atrazine from water, wastewater, and contaminated soil, e.g. incineration, thermal desorption, UV, peroxides, metal oxides, reverse osmosis, and electrodialysis (Rodrigo et al. 2014). These treatments are generally expensive, may involve the formation of toxic by-products, and in some cases the end products require further treatment. Bioremediation is a cost-

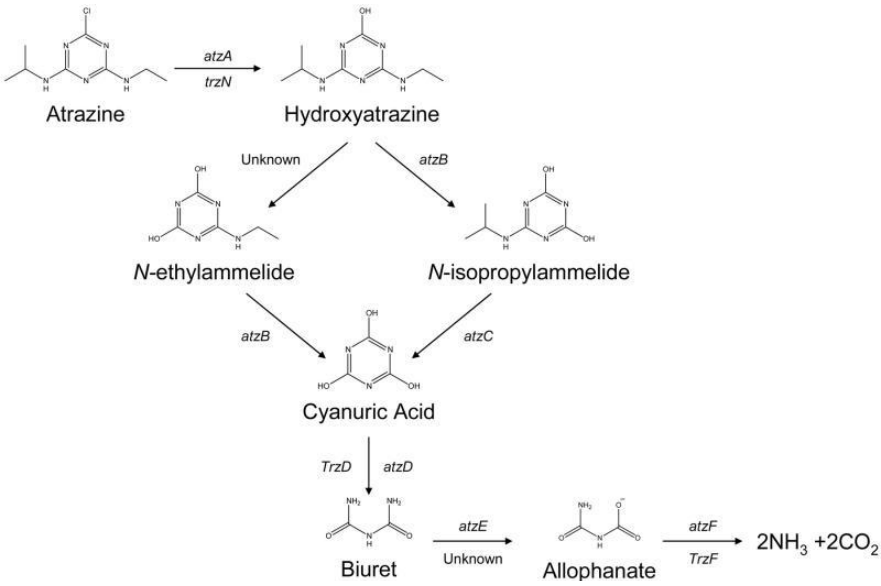

Fig. 2 The bacterial metabolic pathway of atrazine, Krutz et al. (2008).

effective and environmentally friendly alternative.

The natural dissipation of atrazine in soil is influenced by biotic and abiotic processes. The clay and organic matter content of an environment are related to the extent of adsorption of atrazine in soil and sediment particles (Nemeth-Konda et al. 2002). Such an adsorption process enables the accumulation of atrazine and decreases its bioavailability. Microbes in the soil are the primary degraders of atrazine. The biodegradation of atrazine in soil is space-variable, being slower in subsurface zones than in surface soil. Under the vadose zone and subsurface aquifer conditions, low temperatures and a lack of degrading organisms are likely to be primary factors limiting atrazine biodegradation (Radosevich et al. 1996).

Krutz et al. (2008) documented the bacterial metabolic pathway of atrazine (Fig. 2). Currently known atrazine-degrading isolates are taxonomically diverse, including members from the genera *Acinetobacter* (Singh et al. 2004), *Agrobacterium* (Struthers et al. 1998) *Arthrobacter* (Zhang et al. 2011), *Chelatobacter* (Cheyns et al. 2012), *Delftia* (Vargha et al. 2005), *Nocardioides* (Omotayo et al. 2012), *Pseudaminobacter* (Topp et al. 2000), *Pseudomonas* (Hernández et al. 2008), *Rastonia* (Stamper et al. 2002), *Rhizobium* (Bouquard et al. 1997), and *Rhodococcus* (Behki et al. 1993). Of these, the *Pseudomonas* sp. strain ADP (Mandelbaum et al. 1995) has become a reference strain and has been used to elucidate the sequences of the catabolic enzymes involved in aerobic

degradation pathways and to develop probes for the genes encoding these enzymes. The characterization of atrazine-degrading bacterial strains revealed the presence of *atzABCDEF* genes in plasmid and the encoding enzymes involved in the oxygen-dependent degradation of atrazine (de Souza et al. 1996; Martinez et al. 2001). Further studies revealed that these genes were highly conserved in different microbial genera and their worldwide spread suggested a potential molecular mechanism for the dispersion of the *atzABC* genes to other soil bacteria (de Souza et al. 1998). In addition, two other atrazine-degrading genes encoding the enzymes atrazine chlorohydrolase and cyanuric acid hydrolase were characterized as *trzN* and *trzD*, respectively (Karns 1999; Mulbry et al. 2002).

Compared to the intensive studies on microbial atrazine degradation and its molecular mechanisms, the attention paid to the microbial degradation of terbutryn is limited. Only a few studies have been carried out on the topic. The ability to degrade terbutryn has been reported for strains from the genus *Acinetobacter* (Singh et al. 2004). Terbutryn biodegradation by strains of the genera *Arthrobacter* or Pseudomonas in a lab-scale biofilm reactor was reported, but the degradation pathway remained unclear (Sánchez-Sánchez et al. 2013).

1.4 Herbicide effects on microbial communities in soil and groundwater

Microbial communities play key roles in the functions of natural ecosystems, such as primary production, organic matter decomposition, nutrient cycling, and the natural attenuation of contaminants and they thus contribute to soil and water purification processes by providing essential ecosystem services. Our estimation of microbial community distribution has been governed by the dictum "Everything is everywhere, but the environment selects" (De Wit and Bouvier 2006). Microbial distribution is determined almost entirely by the selection generated by the prevailing abiotic and biotic conditions of growth (Koskella and Meaden 2013). Selection can be a significant factor in structuring natural microbial communities.

Many factors influence the structural composition and diversity of the soil microbial community, especially its functional diversity. These factors can be roughly divided into two categories, natural and man-made factors. Natural factors include vegetation, soil type, temperature, moisture, and pH value. Man-made factors include human management of soil, e.g. pesticides, fertilizers, and soil tillage (Zhou et al. 2007). Soil microorganisms are varyingly affected by pesticides, including herbicides, fungicides, and insecticides. They may produce different degrees of soil microbial inhibition, reduce microbial diversity and biomass, and affect soil microbial community structure and functional changes. Hu et al. (2005) studied the effects of atrazine on the soil microbial community. Results showed that the application of atrazine (50 mg kg^{-1}) to soil increased the soil respiration rate significantly and reduced bacterial community diversity. The application of atrazine obviously stimulated soil

microorganisms. Atrazine could shift the microbial community structure and function significantly (Seghers et al. 2003). According to the denaturing gradient gel electrophoresis (DGGE) method, atrazine can alter bacterial community structure (Briceño et al. 2010). Chen et al. (2015) observed that atrazine had significant inhibitory effects on soil net nitrogen nitrification in a 28-day microcosm incubation, and the soil microbial community was significantly affected during the incubation.

The list of atrazine-degrading bacteria is long and bacterial degradation pathways of atrazine are well described (Verma et al. 2014), while information of the impact of atrazine on soil bacterial communities remains rare. Herbicides may have immediate or long-term toxicity effects on bacteria. The effects of herbicides on soil microbial communities are conventionally studied by techniques based on microbial cultivation. For example, the application of atrazine can inhibit the bacterial population in soil. Repeated field applications of atrazine increase the rate of mineralization and may cause an accumulation of the chemical to a point that may have deleterious effects on soil microbiological and biochemical activities. Several microorganisms able to remove atrazine from soil by degrading it have been enriched and isolated (Singh et al. 2004; Getenga et al. 2009). However, most of the studies have been conducted in the temperate and tropical zones, and more attempts to investigate the microbial degradation of triazine pesticides in the boreal area should be made.

The occurrence of bacterial populations able to remove atrazine does not exclude the risk of chronic contamination, which can negatively affect the most sensitive natural populations and, consequently, decrease bacterial diversity and to some extent ecosystem functioning. The high atrazine input can induce the spread of degradation genes in the soil microbial community (Udiković-Kolić et al. 2011). Long-term application of atrazine can induce some soil microbial adaptation. These microbial populations can utilize atrazine as carbon and nitrogen sources, and the high concentrations of atrazine can provide more carbon and energy for their growth (Smith et al. 2005; Shapir et al. 2007).

Groundwater environments lack light, have a low availability of organic carbon and nutrients, and low constant temperature and oxygen levels. These factors are among the most important to surface soil and aquatic microbial communities. Physical and chemical conditions in the subsurface environment strongly influence not only the microbial community but also the behavior of herbicides (Park et al. 2009; Nousiainen et al. 2015). Herbicides are usually present at low concentrations in groundwater. Low organic content and a shortage of degrading microbes are the main reasons for the low natural mineralization rate of triazine herbicides. Nousiainen et al. (2015) claimed that natural mineralization of atrazine does not occur in Finnish subsurface soils, because the nutritional conditions do not favor degradation. The concentration of atrazine present in boreal groundwater is low. Atrazine concentration in groundwater deposits is positively correlated with organic matter, nitrogen, and lead (Talja et al. 2008).

1.5 Data from cultivations and advanced approaches

Selective enrichment favors bacteria with the capacity to grow under special conditions, e.g. certain bacteria can grow in the presence of carbon and/or nitrogen sources such as triazine pesticides. Carbons and nitrogens of the aminoalkyl groups and nitrogens of the triazine rings can theoretically be utilized as growth substrates for bacteria during biodegradation (Bichat et al. 1999). There is a very long history of "plating technology" in the isolation of bacteria from environmental samples. Although many bacterial species grow on media, the majority remain uncultivable even under optimized conditions. For this reason properly studying the bacterial diversity of environmental samples has been very difficult (Youssef et al. 2015).

The development of chemical and molecular technologies, such as phospholipid fatty acid (PLFA) and 16S rDNA DGGE enables us to examine the world of uncultivable bacteria. Microbial ecology can be studied by comparing profiles of bacterial communities, which is not as precise as species-based ecological diversity. In recent decades, Sanger DNA sequencing technology has developed rapidly (Sanger and Coulson 1975; Nyrén 2007; Liu et al. 2012). As a result, the number of bacterial 16S rDNA sequences in databases is increasing exponentially. The Ribosomal Database Project (RDP) II databases host a collection of 10244 bacterial rRNA gene sequences in the training set Version 14 released in May 2015 (Cole et al. 2005). The classification of bacterial colonies on medium plates has become easier and more precise with Sanger DNA sequencing of 16S rDNA. Visualizing bacterial diversity and community structure was possible for the first time with Sanger sequencing and cloning technology (SC), and bacterial 16S rDNA libraries containing thousands of sequences could be obtained. However, due to the low efficiency of Sanger sequencing, SC was very expensive to use. In 2005, Roche developed the first next-generation sequencer (454 Life Sciences, Roche Diagnostics, CT, USA) based on pyrosequencing technology. Since then, it has become widely used in microbial ecology studies. In 2015, the newest Roche 454 GS FLX+ System was able to read sequence lengths up to 1000 bp, and generate approximately one million sequences per run with a consensus accuracy of 99.997% in 23 hours.

The fast development of next-generation sequencing technology does not mean that Sanger sequencing and plating is useless. The sequencing technology only gives information of DNA. Plating is the only way if isolating and cultivating bacteria is necessary. Only isolated bacterial strains with Sanger sequencing of 16S rDNA can be considered reliable when adopted by e.g. RDP databases. In addition, if the full length of 16S rDNA is needed, Sanger sequencing is the only convenient method eligible, as none of the next-generation sequencing

technologies are able to reach accuracies of 1400 bp, which is the accuracy level achievable with Sanger sequencing.

2 Objectives of the study

This thesis focused on studying the microbial ecology of degradation of the pesticide 2-chloro-4-ethylamino-6-isopropylamine-s-triazine (atrazine) and 2-t-butylamino-4-ethylamino-6-methylthio-s-triazine (terbutryn). The pesticide problem in groundwater in Finland is mainly due to urban activities, and surface soils and groundwater sedimentary deposits were collected in the city of Lahti and studied in a laboratory. The influences of water flow, indigenous microbes, additional carbon source, and physicochemical conditions of soils and deposits on the degradation of atrazine and terbutryn were studied. Several bacterial strains degrading atrazine or terbutryn were isolated and identified. The aim was to find the optimal conditions for the microbial degradation of atrazine and terbutryn, and to isolate bacterial strains with possible potential in bioremediation.

The overall objective of the research is to use information obtained for the microbial remediation of groundwater resources contaminated with triazines, using atrazine and terbutryn as model compounds. Microbial community compositions were determined and compared in atrazine-contaminated environments including farmland, atrazine-treated reforested farmland, primary forest, garden surface soils, and subsurface sediments/deposits from drilling sediments and groundwater monitoring pipes. The effects of environmental physicochemical composition on atrazine dissipation were determined. The results of this thesis will provide information on optimizing biotic and abiotic conditions for pesticide bioremediation.

The hypotheses of this thesis are 1) Microbial community compositions differ between clean and contaminated environments, and between surface and subsurface environments. 2) Environmental physicochemical composition can be related to microbial community composition and pesticide bioremediation.

3 MATERIALS AND METHODS

3.1 Origins of studied soils, sediments, and deposits

Samples for the mesocosm experiments were collected from the city of Lahti (60° 58' 0" N / 25° 40' 0" E, Finland) with approximately 100 000 inhabitants. (i) Surface soils were collected from railway C, gardens E and F, and flowerbed G., Simazine was used two years prior to sampling in garden point F and flowerbed G, and garden point E was located 100 m from point F. (ii) The subsurface deposits below the groundwater table were collected from groundwater monitoring pipes A, B, C, and D using a Waterra pump HL 21507 (Ontario, Canada) equipped with an aggregate power 2601 BV (2.5KW, Hollola, Finland). The sediments at the bottom of the groundwater well were collected using an Ekman grab sampler (Duncan and Associates, Cumbria, UK). Sampling pipes B and C contained atrazine in groundwater, while the water in pipes A and D was clean (Talja et al., 2008). The water-deposit slurries were allowed to settle, the water was removed, and the deposits were transferred to plastic bags (article II). (iii) The drilling sediments were collected from a depth of 13.6 m (groundwater table 14.0 m) using a drill of 4.8 cm in diameter (Tieliikelaitos, Finland), and the sediments were transferred to plastic bags (article III). The drilling place was located close to pipe C, which contained atrazine in groundwater.

The study sites in China were located on a forested plain in Wuying (article I), north-eastern China (129° 11' 16.42" E, 48° 09' 47.24" N). Soil samples were taken from (i) a conifer and broadleaf mixed primary forest, (ii) a secondary forest that was a reforested agricultural field with mainly Korean pine planted in the early 1990s; and (iii) farmland mainly used for the cultivation of corn. Atrazine has been applied to the farmland annually (1500–1875 g ha^{-1})

3.2 Experimental setup

3.2.1 Isolation of *Pseudomonas fluorenscens* group strains

The 15-g (dry weight) sample or sterilized counterpart in a 100 ml flask was supplemented with atrazine (100 µg g^{-1}) and terbutryn (67 µg g^{-1}, not in drilling sediments), all in triplicate (Table 1 article II). Surface soil moisture was adjusted to 60% of the saturated soil, and groundwater deposits/sediments were supplemented with 50 ml of water. Approximately 100 mg samples were collected at intervals from the soil mesocosms, and 100 µl samples were collected from the slurries. To isolate Pseudomonas spp., 100 µl was cultivated on mineral agar (Pukkila et al., 2009; atrazine, 33 mg l^{-1}; or terbutryn, 20 mg l^{-1}) from 100 mg of soil in 1.2 ml of water; deposit/sediment slurry; and serially diluted drilling sediment slurry

Table 1. Physical and chemical analyses to determine the experiment conditions used in this thesis.

Analysis	Purpose of the method	Manufacture/Reference	Article
NH_4-N, NO_3-N, Total-C, Total-N, Total-P, Total-K	To determine nutrient levels in soils, groundwater deposits, and drilling sediments	Parkinson and Allen, 1975 Bremner and Tabatabai, 1972 SFS 5505, SFS-EN ISO 10304−1, SFS-EN ISO 10304−2.	I, II
Elements Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn	To specify the type of elements present in soils, groundwater deposits, and drilling sediments	SFS-EN ISO/IEC 17025	II
Dry weight and organic matter	To determine the amount of water in the soils	SFS-EN 13040 SFS-EN 13039	I,II,III
pH	To determine the acidity or alkalinity of soils	Metler Toledo MP220 pH meter	I
Temperature	To monitor the temperature of a laboratory for different mesocosms		II,III
Atrazine/ terbutryn extraction	To extract herbicides	Liu et al. (2015)	I,III
Atrazine/ terbutryn concentration	To determine atrazine / terbutryn concentration in soils, groundwater deposits, and drilling sediments.	HPLC	I,II,III

3.2.2 Atrazine degradation in stagnant and circulating water sediments

To test the effects of physical, chemical, and microbial factors on atrazine degradation in drilling sediments, atrazine dissipation was studied (a) in sediment slurries (modeling stagnant groundwater), and (b) during water flow through sediment columns (modeling groundwater flow through sediments). Both experiments consisted of eight different treatments as presented in article III. (a) In the sediment slurries, atrazine dissipation was followed for ten days in experiments performed in triplicate. Sediments (15.0 g dry weight) in 50 ml of sterilized water were shaken in 100-ml flasks (150 rpm, Laboshake, Gerhardt, Königswinter, Germany). (b) In the sediment column samples collected in duplicate, atrazine dissipation was followed for ten days during water circulation through the saturated sediment columns. To prepare the columns, 10 ml sterilized syringes were filled with 14.7 g (dry weight) of sediments, and 160 ml of liquid was circulated through the sediment columns at a flow rate of 16.7 ml min^{-1} using pipes connected to a multichannel pump (ISM 404B, Ismatec, Germany). The liquids in the flasks were shaken at 150 rpm (Unimax 1010, Heidolph Instruments).

3.3 Analyses

Soil properties including pH, temperature, organic matter, total-K, total-P, total-N, NH_4-N, NO_3-N, dry weight, and elements (Mn, Zn, Pb, Cd, Co, Cr, Ni, Cu, and Fe) were analyzed as presented (Table 1). Concentrations of atrazine and terbutryn were analyzed using high performance liquid chromatography (HPLC). More precise methods for pesticide analyses are presented in articles I, II, III. Bacterial cultivation, identification of microbes by 16S rDNA sequencing, and sequence analyses were performed as listed in Table 2.

DNA was extracted from soil or bacterial strains for the DNA-based analyses, and then used as a template to amplify the 16S rDNA sequence by polymerase chain reaction (PCR) with general bacterial 16S rRNA gene primers (Edwards et al. 1989). Bacterial strain sequences were compared with databases using the FASTA program, and phylogenetic clustering was determined. The DNA sequences generated by 454-pyrosequencing were analyzed using MOTHUR (v1,35,0, 64-bit for Windows) according to standard operating procedures (Schloss et al. 2011). Phylogenetic diversity was determined in MOTHUR based on a phylip-formatted distance tree of unique sequences.

3.4 Calculations

Rarefaction, Chao 1 diversity index, Shannon's diversity index, and evenness (Simpson's complement) were estimated for each sample using Estimates (version 8.2, Colwell, 2006). An indicator species analysis was performed based on the 200 most frequent operational taxonomic units (OTUs) using the multipatt function in the indicspecies package (De Caceres and Jansen, 2015) in R. The SPSS Statistical package for Windows (SPSS Inc., Chicago, IL, USA) was used to calculate the Pearson two-tailed correlation analyses (II, III), analysis of variance (ANOVA) (II), and principal component analysis (PCA) (II). ANOVA was followed by the Tukey's test or the nonparametric Kruskal-Wallis test ($p < 0.05$), followed by pairwise comparisons using the Mann-Whitney test (Kruskal and Wallis 1952; Mann and Whitney 1947; II, III).

Table 2. Microbial and molecular methods used in this thesis. PCR, polymerase chain reaction. More precise descriptions of methods are presented in articles I–III.

Analysis	Purpose of the method	Equipments/ materials	reference
Enrichment culture	To enrich microbes in mesocosms	Shaker (120 rpm; Unimax 1010, Heidolph Instruments, Schwabach, Germany)	II
Microbial cultivation	To grow microbes on a selective medium as the primary step in isolating microbes		I,II,III
DNA-extraction	To extract DNA from a microbial culture as the first step in characterizing microbes	UltraClean™ Microbial DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA)	I,II,III
PCR	To amplify microbial DNA	DNA Engine DYAD Peltier Thermal Cycler (Bio-Rad, Hercules, CA, USA)	I,II,III
Electrophoresis	To detect PCR products and estimate DNA concentration	Chemi Imager 5500 (Alpha Innotech Corporation, San Leandron, CA, USA)	
Phylip package	To conduct phylogenetic analyses	Felsenstein 1989	II
Fatty acid analyses	To determine the fatty acid composition of bacteria from mesocosms	(Suutari et al. 1990; Suutari and Laakso 1992)	II
Staden package	To assemble, edit and analyze DNA sequences	(Bonfield et al. 1995)	II
Sequencing and DNA sequence analyses	To identify the microbe based on the 16S rRNA genes	DNA Sequencing Laboratory (Institute of Biotechnology, University of Helsinki, Helsinki, Finland)	I,II,III

Table 3. Programs used in this thesis for statistical analyses and microbial diversity analyses. More precise descriptions of methods are presented in articles I–III.

Program	Purpose	Reference	Article
Arlequin	To analyze population genetics data of isolated microbes	(Excoffier and Lischer 2010)	II
SPSS, Kruskal-Wallis test, Mann-Whitney's test	To conduct principal components analysis	IBM 2013	I,II,III
Mothur	To analyze all sequences generated by 454-pyrosequencing	v1,35,0, 64-bit for Windows	I
Estimates	To estimate Rarefaction, Chao 1 diversity index, Shannon's diversity index, and evenness (Simpson's complement)	version 8.2, Colwell, 2006	I
Unifrac	To calculate phylogenetic diversity of bacterial community in soils		I

4 RESULTS AND DISCUSSION

4.1 Bacterial community composition in surface soils

The ecological effect of reforesting the atrazine-treated farmland was determined by means of massive DNA sequencing technology. Using 454-pyrosequencing we retained 10 225, 13 326, and 11 981 bacterial 16S rRNA gene sequences from farmland, forest soils, and reforested farmland in northeast China, respectively. The obtained pyrosequencing data set represented a diverse soil bacterial community of 1536 OTUs (excluding 4989 global 235 singletons). Bacterial OTUs representing Proteobacteria were the most abundant group, covering approximately 37.6% of total sequence frequency. Apart from the unclassified bacteria, other major detected phyla were Acidobacteria (11.3%), Bacteroidetes (11.1%), Actinobacteria (10.2%), Firmicutes (8.2%), Planctomycete (2.6%), and Verrucomicrobia (2.5%) (Table 2, article I). Bacterial community shifts were observed in the atrazine-treated farmland compared to forest soils (reforested farmland and primary forest), largely attributable to responses of the following taxa: an increase in Actinobacteria, β-proteobacteria, and Firmicutes and a decrease in Acidobacteria and Verrucomicrobia (Table 2, article I). Other major taxonomic groups either remained unchanged across the different land uses or were not frequent enough to be analyzed. The effects of pesticides on soil microorganism communities have been previously documented (Dewey et al. 2012). Soil microbial biomass and enzyme activities decreased significantly, affected by atrazine in agricultural soils at concentrations of 2mg g^{-1}. An atrazine load of 10 mg kg^{-1} reduced microbial diversity, the Shannon-Wiener index (H) values were reduced to 2.23 compared with the control (H = 2.59), within the 28-day incubation time (Chen et al. 2015). However, during this study we were unable to detect any significant diversity level differences in atrazine-treated farmland soils compared to forest soils. The reason behind this might be the low concentration of atrazine (18.32 ± 8.59 μg kg^{-1}) in the studied farmland (article I).

The frequencies of Actinobacteria and Proteobacteria showed positive correlations with atrazine concentrations in soils. These two phyla have also been reported as the most abundant phyla in soils containing agrochemicals (Udiković-Kolić et al. 2010; Godoi et al. 2014). Indeed, many bacterial atrazine degraders are from these two phyla (Udiković-Kolić et al. 2012). In this thesis, we assumed that the degrading microorganisms were initially present in the farmland soils and that they were able to metabolize atrazine. This assumption was based on the fact that the studied farmland sites had been previously treated with atrazine under normal agricultural practices. Land use history has a strong impact on the soil bacterial community (Jangid et al. 2011). It is usually admitted that the exposure of soil to a herbicide promotes the abundance and activity of atrazine-degrading microorganisms (Martin-Laurent et al. 2003). The repeated application of atrazine may have led to growth-dependent atrazine degradation and population increases of selected OTUs capable of metabolizing the contaminant. The observed trend was that atrazine-treated farmland soil was enriched in bacterial groups that were reportedly to

tolerate or utilize atrazine, while the growth of several acidobacterial OTUs appeared to be inhibited. The natural attenuation for 20 years is not enough to degrade atrazine completely in soil (Article I).

4.2 Microbial isolation from soils and subsurface sediments/deposits under pesticide stress

4.2.1. Atrazine and terbutryn degradation in surface soils and subsurface deposits/sediments

Degradation experiments were performed with atrazine and/or terbutryn as nitrogen sources to enrich a bacterial consortium capable of degrading atrazine (Fig. 3). Microbial atrazine degradation was observed in surface soils from gardens E and F, and flowerbed G (Fig. 3a). On day 189, these three soils contained less atrazine than their sterilized counterparts. In subsurface deposits, atrazine degradation was only observed in pipe A deposits, in which atrazine was completely degraded after 176 days, while the degradation of atrazine was negligible in sterilized pipe A deposits (Fig. 3c and article II).

The differences in terbutryn degradation between sterilized and non-sterilized samples were clear. In the soils from garden F and flowerbed G, most of the added terbutryn was dissipated in 189 days, while barely any degradation was observed in the sterilized samples (Fig. 3b). Deposits from pipe A and pipe C showed terbutryn degradation during 400 days (Fig, 3d). Both atrazine and terbutryn degradation was observed in pipe A deposits. An adapted microbial population able to degrade these herbicides may have developed. Terbutryn was reported to be degraded more rapidly than atrazine in the

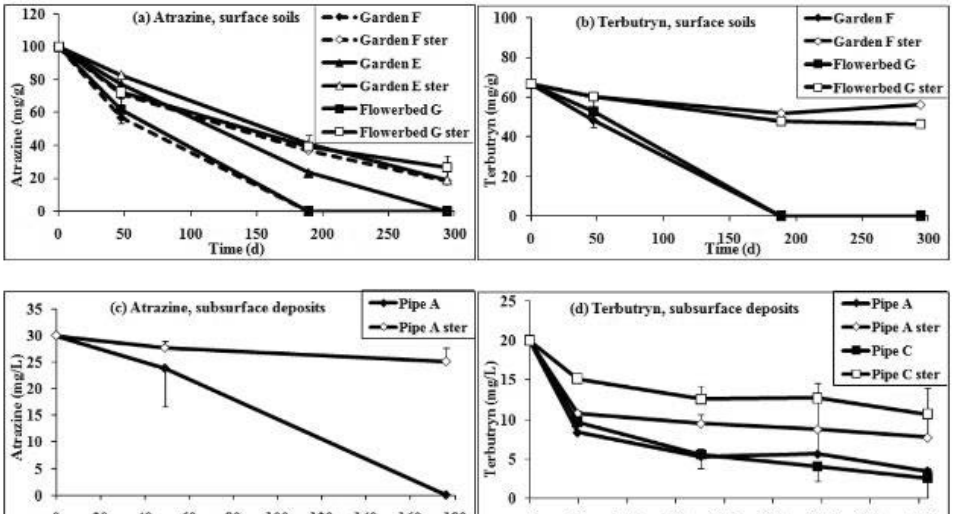

Fig. 3 Atrazine and terbutryn enrichment cultures of (i) surface soils from garden E, garden F, and flowerbed G. (ii) subsurface deposits from pipe A and pipe C.

subsurface deposits (Talja et al. 2008). The C−S bond of terbutryn may break more easily than the C−Cl bond of atrazine. Cl-triazines such as atrazine have formed bonds with organic matter, which were stronger than those of S-triazines such as terbutryn, indicating that sorption may inhibit atrazine degradation more than terbutryn degradation (Abdelhafid et al. 2000). The photodegradation of terbutryn has also been faster than that of atrazine (Lányi and Dinya 2003). The complex, poorly degradable humic substances, typical for the northern boreal region, may form interactions with pesticides and prevent both chemical and microbial degradation.

A laboratory-scale model system of groundwater sediments/deposits was constructed. Atrazine dissipation and related microbial growth were followed in short-term ten-day experiments using two subsurface sediment systems: the stagnant sediment slurries and water circulation in sediment columns. In addition to (1) control sediments (no additives), the following amendments were included in the study arrangements: (2) *Pseudomonas* ADP; (3) four microbes; (4) atrazine; (5) atrazine and *Pseudomonas* ADP; (5) atrazine, *Pseudomonas* ADP, and citrate; (7) atrazine and four microbes; (8) atrazine, four microbes, and citrate (article III, Table 1). The concentrations of organic matter and inorganic elements in the sediments were low, with NH_4-N and NO_3-N levels below the limit of detection (Table 3, article III). According to the two-factor Kruskal-Wallis test ($p < 0.05$), the mode of system (slurry/column) and treatments significantly affected both atrazine dissipation and microbial growth.

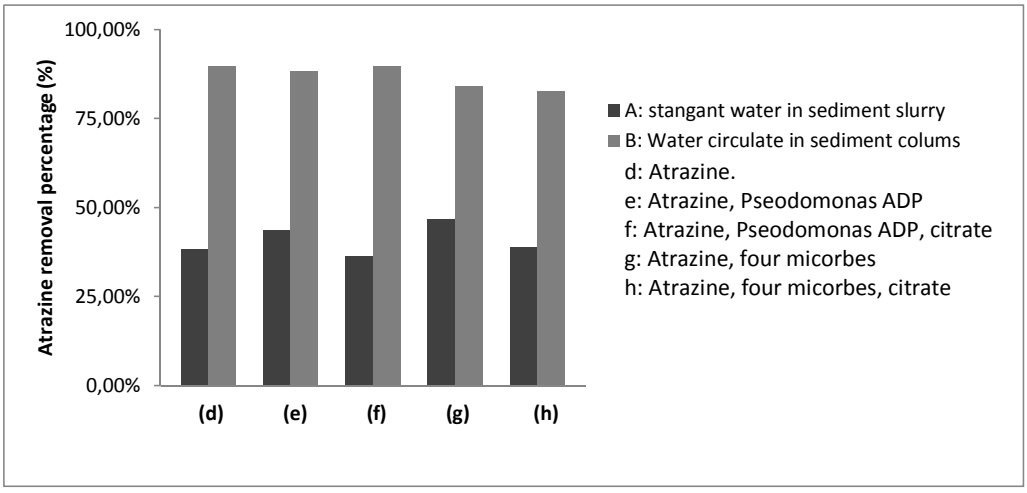

Fig. 4 Atrazine degradation in stagnant sediment and circulated water.

The atrazine concentration decreased from 53.4–63.8 mg l^{-1} in the sediment slurry down to 10.3–17.5 mg l^{-1} in the circulating water of columns (article III, Fig. 4). At the end of the experiments, no atrazine was detected in the extraction of the column sediments. The addition of *Pseudomonas* ADP, four microbes, or citrate had minor effects on the atrazine concentration and cell numbers of slurries during the ten-day experiment. At the end of the experiment, the quantity of atrazine extracted from the atrazine-amended sediments in the slurries was 50.1±25.8 µg (equivalent to 1.0±0.5 µg l^{-1} of slurry and 3.3±1.7 µg g^{-1} of sediment). The physicochemical conditions of groundwater are very different from soil. The microbial population is approximately 104-fold less than in topsoil. The water temperature remains constantly below 10 ºC. Oxygen and the amount of dissolved organic matter (DOM) are low. All these are essential factors for bacterial growth, and consequently bacterial activity and growth are extremely limited in groundwater environments.

4.2.2 Microbe isolation from surface soils and subsurface deposits/sediments

In this thesis, microbes possibly related to atrazine or terbutryn degradation were enriched in a laboratory, and similar phyla were also increased in atrazine-treated farmland (article I). A total of 37 *Pseudomonas* strains were isolated from mesocosms on mineral agar with atrazine or terbutryn as a nitrogen source (Table 1). The mesocosms were prepared from (i) surface soils, (ii) groundwater deposits slurries, and (iii) drilling sediment slurries. In addition, mesocosms included (iv) sterilized surface soils (indoor soils) and (v) sterilized groundwater deposit slurries (indoor deposits), which were self-colonized indoors during long 599–625-day incubation periods. Atrazine-degrading strains were also isolated from mesocosms to evaluate their potential role in atrazine dissipation. Fifteen strains were isolated from a mineral medium containing terbutryn, and 22 strains were isolated from a mineral medium containing atrazine.

According to pairwise FST values, Pseudomonas communities growing under atrazine stress in five study mesocosms were equivalent to those under terbutryn stress. Further, a total of 17 microbes were isolated from the degradation experiment (article III) on a medium with atrazine as the sole nitrogen source. These isolates represent aerobic strains able to grow as major cultivable bacteria under atrazine stress in the studied subsurface sediment systems with low concentrations of organic matter, NH_4-N, NO_3-N, and elements (Tables 2 and 4 in article III). Zhou et al. (2002) found that carbon plays an important role in shaping soil microbial communities. The observed microbial communities differed between surface and subsurface soils in low carbon conditions, but showed a uniform diversity pattern in high carbon conditions, regardless of depth. The isolated Pseudomonas strains from surface soils and subsurface sediments/deposits differed despite belonging to the same genus (Table 4). The distribution of these isolates was determined

by organic matter, NH_4-N, NO_3-N, and elements, regardless of whether they were enriched in atrazine or terbutryn (Article II). Understanding the forces that shape soil microbial communities should help in properly maintaining soil communities and in the wise use of microbial strains in remediation.

Table 4. Isolated strains from surface soils, groundwater sediments/deposits

		Surface soils	Sterilized surface soils	Sterilized groundwater deposits	Groundwater deposits	Drilling sediment
P. mandelii CIP 105273^T	AF058286	Atr	Atr			
		Ter		Ter		
P. marginalis ATCC 10844^T	AB021401	Atr				
P.veronii CIP 104663^T	AF064460	Atr	Atr	Atr	Atr	
		Ter	Ter			
P. sp. IC038	U85869			Atr		
				Ter	Ter	
*P.*sp.LAB-23	AB051699					Atr
					Ter	

Of the isolates gathered from the drilling sediments, three strains were Gram-positive Actinobacteria from the genera *Rhodococcus* (ATR50), Streptomyces (ATR42), and *Williamsia* (ATR44), and the rest were from the phylum Proteobacteria (Table 5). The isolates from class alphaproteobacteria belonged to the genera *Methylobacterium* (ATR47, ATR52) and *Sphingomonas* (ATR64); isolates from class betaproteobacteria belonged to the genera *Burkholderia* (ATR49) and *Variovorax* (ATR46, ATR63), and isolates from the class gammaproteobacteria belonged to the genera Acinetobacter (ATR55) and *Pseudomonas* (ATR41, ATR45, ATR51, ATR53, ATR54, ATR56, ATR62). Actinobacteria and Proteobacteria were also the dominant phyla

in the atrazine-treated farmland based on the bacterial community analyses (article I). All of the isolates in this thesis gathered from Finnish urban soils and subsurface sediments/deposits belonged to the same two phyla. The sampling sites in China and Finland are both located in the boreal region, and the black soils in northeast China and Finland are rich in nutrients and organic matter (Liski and Westman 1997). Similar conditions may uphold similar microbial communities.

Table 5. Comparison of phyla and genera of the 54 isolated bacterial strains (surface soil, subsurface deposits/sediments, and cultivation) and indicator species of atrazine-treated farmland bacterial communities based on analysis of the 200 most frequent OTUs using 454-pyrosequencing.

Atrazine-treated farmland surface soils		Urban surface soils and subsurface sediments/deposits	
Phylum	Genus	Phylum	Genus
Actinobacteria	*Arthrobacter*	Actinobacteria	*Williamsia*
Actinobacteria	*Streptomyces*	Actinobacteria	*Streptomyces*
Actinobacteria	*Rhodococcus*	Actinobacteria	*Rhodococcus*
Proteobacteria	*Methylobacterium*	Proteobacteria	*Methylobacterium*
Proteobacteria	*Pseudomonas*	Proteobacteria	*Pseudomonas*
Proteobacteria	*Ralstonia*	Proteobacteria	*Acinetobacter*
Proteobacteria	*Rhodanobacter*	Proteobacteria	*Burkholderia*
		Proteobacteria	*Sphingomonas*
		Proteobacteria	*Variovorax*

Members of the genus *Arthrobacter* have been reported to be dominant among clones in the 16S rRNA gene clone library of atrazine-mineralizing bacterial communities originating from the contaminated soils of an agrochemical factory (Udikovic-Kolic et al. 2010). In addition, one *Arthrobacter* strain was isolated from farmland in Heilongjiang, China. It contained the degrading genes *trzN*, *atzB*, and *atzC*, which enable the strain to decompose atrazine to cyanuric acid (Zhang et al., 2011). These atrazine degradation genes are generally highly conserved and located on a plasmid, which increases the probability of spreading the gene cluster between bacteria independent of their genera

(Sajjaphan et al. 2004; Martin-Laurent et al. 2006; Liu and Parales 2009). The isolated 54 bacterial strains belonged to Actinobacteria and Proteobacteria. These two phyla were also detected in atrazine-treated farmland. The genera *Rhodococcus*, *Pseudomonas, Streptomyces*, and *Methylobacterium* were found among the isolates from Finland and 16S rDNA sequences from China (Table 5). Isolating potential pesticide degradation strains from these genera appears promising. However, isolate diversity within the same genus was high (Table 4). Degradation activity and capability do not assure successful practical application in sediments. The four microorganisms inoculated to the sediments did not grow among the major cultivable microorganisms as in the degradation experiment (article III). Two strains were bacteria (*Pseudomonas* sp. ATR18/2; *Janthinobacterium* sp. ATR17/2) and two were fungi (*Acremonium* sp, ATR16/2; *Penicillium* sp. ATR18/2b). These four microorganisms were isolated from groundwater deposits on selective medium containing atrazine (33 mg l^{-1}). They are indigenous microbes in groundwater deposits, yet they were not among the cultivable strains either in the stagnant or in the water-circulated sediments.

4.3 Remediation of atrazine in soils and groundwater sediments

In this study, atrazine was detected (4.64 ± 2.02 µg kg^{-1}) in reforested farmland approximately 20 years after reforestation, indicating that the contaminant was not completely degraded by natural attenuation. The active farmland soil was annually treated with large amounts of atrazine to control weeds, and was unsurprisingly found to contain 18.32 ± 8.59 µg kg^{-1} of atrazine. Studies showed the presence of atrazine in the soil over 20 years after the last application (Jablonowski et al. 2008).

Atrazine-containing groundwater has been a global problem for a long time (Mirgain et al. 1993). The high persistence of atrazine ring carbon is not completely understood and could be due to a combination of binding to the soil matrix in unavailable forms or lack of metabolic capability. The accumulation of the parent compound in soil may represent a long-term source of dissolved or colloid-bound atrazine and its metabolites to groundwater or surface waters. The bioavailability of pesticides adsorbed to soils is an important factor in determining their environmental fate. Generally, soil-adsorbed atrazine is considered to have low bioavailability for biodegradation (Park et al. 2003). Atrazine can bind to soil, sediment, and/or DOM, consequently reducing its bioavailability (Akkanen et al. 2001). In this thesis, water circulation enhanced atrazine degradation, possibly because the mechanic force produced by flowing water can release atrazine from its bind in sediment particles (article III). Water circulation increases the amount of dissolved oxygen, and consequently the supported organisms grow aerobically (article III). An enhanced biodegradation of triazine may be achieved by increasing the oxygen content in groundwater sediments with pumping strategies.

Many factors have been reported to advance the degradation of triazine herbicides, including previous exposure (Fenoll et al. 2014) and nitrogen scarcity (Sinkkonen et al. 2013). The effects of carbon amendment on triazine degradation are contradictory. The addition of glucose has negatively affected the growth of atrazine-degrading bacteria (Xie et al. 2013), however, atrazine dealkylation was enhanced in treatments with 100 and 1000 μg g^{-1} of sucrose (Ngigi et al. 2012). Little is known of how carbon impacts the added degraders performing bioremediation of soils with high concentrations of s-triazine herbicides. Additional citrate as the major source of carbon and energy did not result in increased atrazine degradation in our study. Fenner et al. (2007) has suggested that increasing organic carbon contents could result in inhibited atrazine degradation rates, most likely due to an enhanced extent of atrazine sorption and hence lower bioavailability. Organic carbon affected the distribution of *Pseudomonas* strains in the soil layers (article II). Organic carbon therefore needs to be adjusted well to provide an optimal microbial chemical condition for pesticide degradation in practical microbial remediation.

5 CONCLUSIONS

The microbial dissipation of atrazine and terbutryn in soils and groundwater sediments/deposits was observed in our laboratory study. Bacterial strains growing in the presence of atrazine or terbutryn were isolated from the studied groundwater sediments/deposits and surface soils. The diversity of atrazine- and terbutryn-degrading microbes in surface soils and groundwater sediments/deposits was relatively high. The isolates belonged to the genera *Acinetobacter*, *Burkholderia*, *Methylobacterium*, *Pseudomonas*, *Rhodococcus*, *Sphingomonas*, *Streptomyces*, *Variovorax*, and *Williamsia*. By means of DNA sequencing, soil bacterial diversity in atrazine-treated surface soils remained unchanged compares to untreated soils, but the bacterial community structure differed from the nearby reforested soil and primary forest. The same phyla were observed by both cultivation-based and DNA sequencing - based methods. They present the potential atrazine degraders.

The long-term 20 years of natural attenuation did not completely degrade soil atrazine residues. In our short-term ten-day laboratory degradation experiment, the atrazine degradation rate in groundwater sediment was 36.2–89.7%. Atrazine bioavailability seems to be the limiting factor for biodegradation. Mechanical forces caused by flowing water in sediments released atrazine for microbial degradation, resulting in improved atrazine degradation. The difference in the microbial communities of surface soils and subsurface sediments/ deposits is significant. By studying the degrading microbes and the optimal conditions for microbial degradation, bioremediation techniques may be developed to clean contaminated sites. In this thesis atrazine and terbutryn were studied as models of the triazine group, where group members shared similar chemical structures. The information obtained can be used in future research to develop solutions for the bioremediation of triazine-contaminated sites.

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7 REFERENCES

Abdelhafid R, Houot S, Barriuso E (2000) Dependence of atrazine degradation on C and N availability in adapted and non-adapted soils. Soil Biology and Biochemistry 32:389-401 doi: http://dx.doi.org/10.1016/S0038-0717(99)00167-4

Alavanja MCR (2009) Pesticides Use and Exposure Extensive Worldwide. Reviews on environmental health 24:303-309

Behki R, Topp E, Dick W, Germon P (1993) Metabolism of the herbicide atrazine by *Rhodococcus* strains. Applied and Environmental Microbiology 59:1955-1959

Bichat F, Mulvaney RL, Sims GK (1999) Microbial utilization of heterocyclic Nitrogen from atrazine. Soil science society of America Journal 63:100-110 doi: 10.2136/sssaj1999.03615995006300010016x

Bonfield JK, Smith KF, Staden R (1995) A new DNA sequence assembly program. Nucleic Acids Research 23:4992-4999 doi: 10.1093/nar/23.24.4992

Bouquard C, Ouazzani J, Prome J, Michel-Briand Y, Plesiat P (1997) Dechlorination of Atrazine by a *Rhizobium* sp. Isolate. Applied and Environmental Microbiology 63:862-866

Briceño G, Jorquera MA, Demanet R, Mora ML, Durán N, Palma G (2010) Effect of cow slurry amendment on atrazine dissipation and bacterial community structure in an agricultural Andisol. Science of The Total Environment 408:2833-2839 doi: http://dx.doi.org/10.1016/j.scitotenv.2010.03.014

Burkhardt M, Zuleeg S, Vonbank R, Bester K, Carmeliet J, Boller M, Wangler T (2012) Leaching of Biocides from Façades under Natural Weather Conditions. Environmental Science & Technology 46:5497-5503 doi: 10.1021/es2040009

Chen Q, Yang B, Wang H, He F, Gao Y, Scheel R (2015) Soil microbial community toxic response to atrazine and its residues under atrazine and lead contamination. Environmental Science and Pollution Research 22:996-1007 doi: 10.1007/s11356-014-3369-7

Cheyns K, Calcoen J, Martin-Laurent F, Bru D, Smolders E, Springael D (2012) Effects of dissolved organic matter (DOM) at environmentally relevant carbon concentrations on atrazine degradation by Chelatobacter heintzii SalB. Applied Microbiology and Biotechnology 95:1333-1341 doi: 10.1007/s00253-011-3741-1

Cole JR, Chai B, Farris RJ, Wang Q, Kulam SA, McGarrell DM, Garrity GM, Tiedje JM (2005) The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. Nucleic Acids Research 33:D294-296

Cooper J, Dobson H (2007) The benefits of pesticides to mankind and the environment. Crop Protection 26:1337-1348 doi: http://dx.doi.org/10.1016/j.cropro.2007.03.022

De Caceres, M., Jansen, F., 2015. "indicspecies" Relationship Between Species and Groups of Sites package for R. Version 1.7.5

de Souza ML, Sadowsky MJ, Wackett LP (1996) Atrazine chlorohydrolase from

Pseudomonas sp. strain ADP: gene sequence, enzyme purification, and protein characterization. Journal of Bacteriology 178:4894-4900

de Souza ML, Wackett LP, Sadowsky MJ (1998) The atzABC genes encoding atrazine catabolism are located on a self-transmissible plasmid in Pseudomonas sp. strain ADP. Applied and Environmental Microbiology 64:2323-2326

De Wit R, Bouvier T (2006) 'Everything is everywhere, but, the environment selects'; what did Baas Becking and Beijerinck really say? Environmental Microbiology 8:755-758 doi: 10.1111/j.1462-2920.2006.01017.x

Dewey KA, Gaw SK, Northcott GL, Lauren DR, Hackenburg S (2012) The effects of copper on microbial activity and the degradation of atrazine and indoxacarb in a New Zealand soil. Soil Biology and Biochemistry 52:64-74 doi: http://dx.doi.org/10.1016/j.soilbio.2012.04.009

Edwards U, Rogall T, Blocker H, Emde M, Bottger EC (1989) Isolation and direct complete nucleotide determination of entire genes – characterization of a gene coding for 16s-ribosomal RNA. Nucleic Acids Research 17:7843-7853. doi: 10.1093/nar/17.19.7843

European Union, Council Directive 98/83/EC on the quality of water intended for human consumption. Official Journal of the European Communities, 3 November, 1998

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564-567 doi: 10.1111/j.1755-0998.2010.02847.x

Fahad S, Hussain S, Chauhan BS, Saud S, Wu C., Hassan S, Tanveera M, Jan A, Huang J (2015) Weed growth and crop yield loss in wheat as influenced by row spacing and weed emergence times. Crop Protection 71:101-108 doi: http://dx.doi.org/10.1016/j.cropro.2015.02.005

Felsenstein, J. 1989. PHYLIP - Phylogeny Inference Package (Version 3.2). Cladistics 5: 164-166.

Fenner K, Lanz VA, Scheringer M, Borsuk ME (2007) Relating Atrazine Degradation Rate in Soil to Environmental Conditions: Implications for Global Fate Modeling. Environmental Science & Technology 41:2840-2846 doi: 10.1021/es061923i

Fenoll J, Vela N, Navarro G, Pérez-Lucas G, Navarro S (2014) Assessment of agro-industrial and composted organic wastes for reducing the potential leaching of triazine herbicide residues through the soil. Science of The Total Environment 493:124-132 doi: http://dx.doi.org/10.1016/j.scitotenv.2014.05.098

Getenga ZM, Dörfler U, Schroll R (2009) Laboratory Degradation Studies of 14C-Atrazine and -Isoproturon in Soil from Sugarcane Cultivated Fields Under Kenyan Tropical Conditions. Bulletin of Environmental Contamination and Toxicology 82:678-682 doi: 10.1007/s00128-009-9672-1

Godoi I, Sene L, Barra Caracciolo A (2014) Assessment of the bacterial community structure in a Brazilian clay soil treated with

atrazine. Annals of Microbiology 64:307-311 doi: 10.1007/s13213-013-0665-2

Hernández M, Villalobos P, Morgante V, González M, Reiff C, Moore E, Seeger M (2008) Isolation and characterization of a novel simazine-degrading bacterium from agricultural soil of central Chile, *Pseudomonas* sp. MHP41. FEMS Microbiology Letters 286:184-190 doi: 10.1111/j.1574-6968.2008.01274.x

Hu, J., Dai, XZ., Li, SP. 2005. Effects of atrazine and its degrader *Exiguobaterium* sp.BTAH1 on soil microbial community. Chinese Journal of Applied Ecology. 8: 138-143

IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.

Jablonowski ND, Koeppchen S, Hofmann D, Schaeffer A, Burauel P (2008) Spatial distribution and dharacterization of long-term aged 14C-labeled atrazine residues in soil. Journal of Agricultural and Food Chemistry 56:9548-9554 doi: 10.1021/jf8017832

Jangid K, Williams MA, Franzluebbers AJ, Schmidt TM, Coleman DC, Whitman WB (2011) Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. Soil Biology and Biochemistry 43:2184-2193 doi: http://dx.doi.org/10.1016/j.soilbio.2011.06.022

Karns JS (1999) Gene Sequence and Properties of an s-triazine ring-cleavage enzyme from *Pseudomonas* sp. strain NRRLB-12227. Applied and Environmental Microbiology 65:3512-3517

Kislev ME, Weiss E, Hartmann A (2004) Impetus for sowing and the beginning of agriculture: Ground collecting of wild cereals. Proceedings of the National Academy of Sciences of the United States of America 101:2692-2695 doi: 10.1073/pnas.0308739101

Koskella B, Meaden S (2013) Understanding bacteriophage specificity in natural microbial communities. Viruses 5:806-823

Krutz LJ, Shaner DL, Accinelli C, Zablotowicz RM, Henry WB (2008) Atrazine dissipation in -triazine–adapted and nonadapted soil from Colorado and Mississippi: Implications of enhanced degradation on atrazine fate and transport parameters. Journal of Environmental Quality 37:848-857 doi: 10.2134/jeq2007.0448

Lányi K, Dinya Z (2003) Photodegradation study of some triazine-type herbicides. Microchemical Journal 75:1-13 doi: http://dx.doi.org/10.1016/S0026-265X(03)00021-3

Lebaron, HM, McFarland JE, Burnside OC. eds.2008. The triazine herbicides- 50 years revolutionizing agriculture. Elsevier. USA

Liski J, Westman C (1997) Carbon storage in forest soil of Finland. 2. Size and regional pattern. Biogeochemistry 36:261-274 doi: 10.1023/A: 1005742523056

Liu L, Li Y, Li S, Hu N, He Y, Pong R, Law, M. (2012) Comparison of Next-generation sequencing systems. Journal of Biomedicine and Biotechnology 2012:11 doi: 10.1155/2012/251364

Liu X, Ecarnot M, Kontro M (2015) The physicochemical conditions of isolation source determine the occurrence of *Pseudomonas fluorescens* group species. Annals of Microbiology: 65 (4): 2363-2377. doi 10.1007/s13213-015-1078-1

Liu X, Parales RE (2009) Bacterial chemotaxis to atrazine and related s-triazines. Applied Environmental Microbiology 75:5481-5488 doi: 10.1128/aem.01030-09

Loos R, Locoro G, Comero S, Contini S, Schwesig D, Werres F, Bolchi M (2010) Pan-European survey on the occurrence of selected polar organic persistent pollutants in ground water. Water Research 44:4115-4126 doi: http://dx.doi.org/10.1016/j.watres.2010.05.032

Martin-Laurent F, Barrès B, Wagschal I, Piutti S, Devers M, Soulas G, Philippot L (2006) Impact of the maize rhizosphere on the genetic structure, the diversity and the atrazine-degrading gene composition of cultivable atrazine-degrading communities. Plant and Soil 282:99-115 doi: 10.1007/s11104-005-5316-3

Martin-Laurent F Piutti S, Hallet S, Wagschal I, Philippot L, Catroux G, Soulas G (2003) Monitoring of atrazine treatment on soil bacterial, fungal and atrazine-degrading communities by quantitative competitive PCR. Pest Management Science 59:259-268 doi: 10.1002/ps.630

Martinez B, Tomkins J, Wackett LP, Wing R, Sadowsky MJ (2001) Complete nucleotide sequence and organization of the atrazine catabolic plasmid pADP-1 from *Pseudomonas* sp. strain ADP. Journal of Bacteriology 183:5684-5697 doi: 10.1128/jb.183.19.5684-5697.2001

Matamoros V, Salvadó V (2013) Evaluation of a coagulation/flocculation-lamellar clarifier and filtration-UV-chlorination reactor for removing emerging contaminants at full-scale wastewater treatment plants in Spain. Journal of Environmental Management 117:96-102 doi: http://dx.doi.org/10.1016/j.jenvman.2012.12.021

Mattsson M, Liu X, Yu D, Kontro M (2015) Depth, soil type, water table, and site effects on microbial community composition in sediments of pesticide-contaminated aquifer. Environmental Science and Pollution Research 22:10263-10279 doi: 10.1007/s11356-015-4224-1

Mirgain I, Schenck C, Monteil H (1993) Atrazine contamination of groundwaters in eastern France in relation to the hydrogeological properties of the agricultural land. Environmental Technology 14:741-750 doi: 10.1080/09593339309385345

Muir DCG, Yarechewski AL (1982) Degradation of terbutryn in sediments and water under various redox conditions. Journal of Environmental Science and Health, Part B 17:363-380 doi: 10.1080/03601238209372327

Mulbry WW, Zhu H, Nour SM, Topp E (2002) The triazine hydrolase gene *trzN* from *Nocardioides* sp. strain C190: Cloning and construction of gene-specific primers. FEMS Microbiology Letters 206:75-79 doi: 10.1111/j.1574-6968.2002.tb10989.x

National Academy of Science, 1969. Insecticides. pp. 360-446 In Insect Pest

Management Control Principles and Animal Pest Control. Vol. Publ. 1695. Washington, D.C.

Nemeth-Konda L, Füleky G, Morovjan G, Csokan P (2002) Sorption behaviour of acetochlor, atrazine, carbendazim, diazinon, imidacloprid and isoproturon on Hungarian agricultural soil. Chemosphere 48:545-552 doi: http://dx.doi.org/10.1016/S0045-6535(02)00106-6

Ngigi AN, Getenga ZM, Dörfler U, Boga HI, Kuria B, Ndalut P, Schroll R (2012) Effects of carbon amendment on in situ atrazine degradation and total microbial biomass. Journal of Environmental Science and Health, Part B 48:40-48 doi: 10.1080/03601234.2012.716727

Nousiainen A, Björklöf K, Sagarkar S, Nielsen J, Kapley A, Jørgensen K (2015) Bioremediation strategies for removal of residual atrazine in the boreal groundwater zone. Applied Microbiology and Biotechnology: 1-11 doi: 10.1007/s00253-015-6828-2

Nyrén P (2007) The History of Pyrosequencing®. In: Walker J, Marsh S (eds) Pyrosequencing® Protocols. Humana Press, pp 1-13

Omotayo AE, Ilori MO, Radosevich M, Amund OO (2012) Metabolism of atrazine in liquid cultures and soil microcosms by *Nocardioides* strains isolated from a contaminated nigerian agricultural soil. Soil and Sediment Contamination: An International Journal 22:365-375 doi: 10.1080/15320383.2013.733444

Park J-H, Feng Y, Ji P, Voice TC, Boyd SA (2003) Assessment of Bioavailability of Soil-Sorbed Atrazine. Applied and Environmental Microbiology 69:3288-3298 doi: 10.1128/aem.69.6.3288-3298.2003

Park J, Sanford RA, Bethke CM (2009) Microbial activity and chemical weathering in the Middendorf aquifer, South Carolina. Chemical Geology 258:232-241 doi: http://dx.doi.org/10.1016/j.chemgeo.2008.10.011

Pimentel D (2009) Pesticides and pest control. In: Peshin R, Dhawan A (eds) Integrated Pest Management: Innovation-development Process. Springer Netherlands, pp 83-87

Quednow K, Puttmann W (2007) Monitoring terbutryn pollution in small rivers of Hesse, Germany. Journal of Environmental Monitoring 9:1337-1343 doi: 10.1039/B711854F

Radosevich M, Traina S, Tuovinen O (1996) Biodegradation of atrazine in surface soils and subsurface sediments collected from an agricultural research farm. Biodegradation 7:137-149 doi: 10.1007/BF00114626

Rodrigo MA, Oturan N, Oturan MA (2014) Electrochemically Assisted Remediation of Pesticides in Soils and Water: A Review. Chemical Reviews 114:8720-8745 doi: 10.1021/cr500077e

Sadler EJ, Sudduth KA, Lerch RN, Baffaut C, Kitchen NR (2014) A simple index explains annual atrazine transport from surface runoff-prone watersheds in the north-central USA. Hydrological Processes 28:210-217 doi: 10.1002/hyp.9544

Sajjaphan K, Shapir N, Wackett LP, Palmer M, Blackmon B, Tomkins J, Sadowsky MJ (2004) *Arthrobacter* aurescens TC1 atrazine

catabolism genes *trzN*, *atzB*, and *atzC* are linked on a 160-kilobase region and are functional in *Escherichia coli*. Applied Environmental Microbiology 70:4402-4407 doi: 10.1128/AEM.70.7.4402-4407.2004

Sánchez-Sánchez R, Ahuatzi-Chacón D, Galíndez-Mayer J, Ruiz-Ordaz N, Salmerón-Alcocer A (2013) Removal of triazine herbicides from aqueous systems by a biofilm reactor continuously or intermittently operated. Journal of Environmental Management 128:421-426 doi: http://dx.doi.org/10.1016/j.jenvman.2013.05.050

Sanger F, Coulson AR (1975) A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. Journal of Molecular Biology 94:441-448 doi: http://dx.doi.org/10.1016/0022-2836(75)90213-2

Schloss PD, Gevers D, Westcott SL (2011) Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. PLoS One 6:e27310 doi: 10.1371/journal.pone.0027310

Seghers D, Verthé K, Reheul D, Bulcke R, Siciliano SD, Verstraete W, Top EM (2003) Effect of long-term herbicide applications on the bacterial community structure and function in an agricultural soil. FEMS Microbiology Ecology 46:139-146 doi: 10.1016/s0168-6496(03)00205-8

SFS 5505. Determination of inorganic and organic nitrogen in waste water. Modified Kjeldahl method. Finnish Standards Association SFS, Helsinki, Finland, 1988, (in Finnish).

SFS-EN ISO 10304−1. Water quality. Determination of dissolved fluoride, chloride, nitrite, orthophosphate, bromide, nitrate and sulphate ions, using liquid chromatography of ions. Part 1: Method for water with low contamination. Finnish Standards Association SFS, Helsinki, Finland, 1995.

SFS-EN ISO 10304−2. Water quality. Determination of dissolved anions by liquid chromatography of ions. Part 2: Determination of bromide, chloride, nitrate, nitrite, orthophosphate and sulphate in waste water. Finnish Standards Association SFS, Helsinki, Finland, 1997.

SFS-EN ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories. Finnish Standards Association SFS, Helsinki, Finland, 2005

SFS-EN 13040. Soil improvers and growing media. Sample preparation for chemical and physical tests, determination of dry matter content, moisture content and laboratory compacted bulk density. Finnish Standards Association SFS, Helsinki, Finland, 2000

SFS-EN 13039. Soil improvers and growing media. Determination of organic matter content and ash. Finnish Standards Association SFS, Helsinki, Finland, 2000.

Swiss patent 226180, Verfahren zur Schädlingsbekämpfung", submitted by J.R. Geigy A.G. 7 March 1940, given March 32, 1943, published June 16, 1943

Shapir N, Mongodin EF, Sadowsky MJ, Daugherty SC, Nelson KE, Wackett LP (2007) Evolution of catabolic pathways: genomic insights into microbial s-triazine

metabolism. Journal of Bacteriology 189:674-682 doi: 10.1128/jb.01257-06

Singh P, Suri CR, Cameotra SS (2004) Isolation of a member of Acinetobacter species involved in atrazine degradation. Biochemical and Biophysical Research Communications 317:697-702 doi: http://dx.doi.org/10.1016/j.bbrc.2004.03.112

Sinkkonen A Kauppi S, Pukkila V, Nan H, Płociniczak T, Kontro M, Romantschuk M (2013) Previous exposure advances the degradation of an anthropogenic s-triazine regardless of soil origin. Journal of Soils and Sediments 13:1430-1438 doi: 10.1007/s11368-013-0742-y

Smith D, Alvey S, Crowley DE (2005) Cooperative catabolic pathways within an atrazine-degrading enrichment culture isolated from soil. FEMS Microbiology Ecology 53:265-273 doi: 10.1016/j.femsec.2004.12.011

Stamper DM, Radosevich M, Hallberg KB, Traina SJ, Tuovinen OH (2002) Ralstonia basilensis M91-3, a denitrifying soil bacterium capable of using s-triazines as nitrogen sources. Canadian Journal of Microbiology 48:1089-1098 doi: 10.1139/w02-113

Struthers JK, Jayachandran K, Moorman TB (1998) Biodegradation of atrazine by *Agrobacterium radiobacter* J14a and use of this strain in bioremediation of contaminated soil. Applied and Environmental Microbiology 64:3368-3375

Suutari M, Laakso S (1992) Unsaturated and branched chain-fatty acids in temperature adaptation of *Bacillus subtilis* and *Bacillus megaterium*. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism 1126:119-124 doi: http://dx.doi.org/10.1016/0005-2760(92)90281-Y

Suutari M, Liukkonen K, Laakso S (1990) Temperature adaptation in yeasts, the role of fatty acids. Journal of General Microbiology 136:1469-1474

Talja KM, Kaukonen S, Kilpi-Koski J, Malin I, Kairesalo T, Romantschuk M, Kontro, MH (2008) Atrazine and Terbutryn Degradation in Deposits from Groundwater Environment within the Boreal Region in Lahti, Finland. Journal of Agricultural and Food Chemistry 56:11962-11968 doi: 10.1021/jf802528a

Tomlin CDS. The Pesticide Manual, 12 ed. The British Crop Protection Council, Surrey, UK. 2000.

Topp E, Zhu H, Nour SM, Houot S, Lewis M, Cuppels D (2000) Characterization of an Atrazine-DegradingPseudaminobacter sp. Isolated from Canadian and French Agricultural Soils. Applied and Environmental Microbiology 66:2773-2782 doi: 10.1128/aem.66.7.2773-2782.2000

Udiković-Kolić N, Devers-Lamrani M, Petrić I, Hršak D, Martin-Laurent F (2011) Evidence for taxonomic and functional drift of an atrazine-degrading culture in response to high atrazine input. Applied Microbiology and Biotechnology 90:1547-1554 doi: 10.1007/s00253-011-3198-2

Udikovic-Kolic N, Hrsak D, Devers M, Klepac-Ceraj V, Petric I, Martin-Laurent F (2010) Taxonomic and functional diversity of atrazine-degrading bacterial communities enriched from agrochemical factory soil.

Journal of Applied Microbiology 109:355-367 doi: 10.1111/j.1365-2672.2010.04700.x

Udiković-Kolić N, Hršak D, Devers M, Klepac-Ceraj V, Petrić I, Martin-Laurent F (2010) Taxonomic and functional diversity of atrazine-degrading bacterial communities enriched from agrochemical factory soil. Journal of Applied Microbiology 109:355-367 doi: 10.1111/j.1365-2672.2010.04700.x

Udiković-Kolić N, Scott C, Martin-Laurent F (2012) Evolution of atrazine-degrading capabilities in the environment. Applied Microbiology and Biotechnology 96:1175-1189 doi: 10.1007/s00253-012-4495-0

Vargha M, Takáts Z, Márialigeti K (2005) Degradation of atrazine in a laboratory scale model system with Danube river sediment. Water Research 39:1560-1568 doi: http://dx.doi.org/10.1016/j.watres.2004.10.013

Velisek J, Stara A, Machova J, Dvorak P, Zuskova E, Prokes M, Svobodova Z (2012) Effect of terbutryn at environmental concentrations on early life stages of common carp (*Cyprinus carpio* L.). Pesticide Biochemistry and Physiology 102:102-108 doi: http://dx.doi.org/10.1016/j.pestbp.2011.11.005

Verma J, Jaiswal D, Sagar R (2014) Pesticide relevance and their microbial degradation: a-state-of-art. Reviews in Environmental Science and Bio/Technology 13:429-466 doi: 10.1007/s11157-014-9341-7

Vuorimaa P, Kontro M, Rapala J, Gustafsson (2007) Torjunta-aineiden esiintyminen pohjavedessä. (Occurrence of pesticides in groundwater). The Finnish Environment 42, Finnish Environment Institute, Helsinki (in Finnish)

Xie S, Wan R, Wang Z, Wang Q (2013) Atrazine biodegradation by Arthrobacter strain DAT1: effect of glucose supplementation and change of the soil microbial community. Environmental Science and Pollution Research 20:4078-4084 doi: 10.1007/s11356-012-1356-4

Youssef NH, Couger MB, McCully AL, Criado AEG, Elshahed MS (2015) Assessing the global phylum level diversity within the bacterial domain: A review. Journal of Advanced Research 6:269-282 doi: http://dx.doi.org/10.1016/j.jare.2014.10.005

Zhang S， Wang HF， Lin P， Luo ZJ ， Zhang FJ. Effect of atrazine on soil urease enzyme activity. doi: 10.3969/j.issn.1006-6500.2015.02.016

Zhang Y, Jiang Z, Cao B, Hu M, Wang Z, Dong X (2011) Metabolic ability and gene characteristics of *Arthrobacter* sp. strain DNS10, the sole atrazine-degrading strain in a consortium isolated from black soil. International Biodeterioration & Biodegradation 65:1140-1144 doi: http://dx.doi.org/10.1016/j.ibiod.2011.08.010

Zhou J, Xia B, Treves DS, Wu LY, Marsh TL, O'Neill RV, Palumbo AV, Tiedje JM (2002) Spatial and resource factors influencing high microbial diversity in soil. Applied and Environmental Microbiology 68:326-334 doi: 10.1128/AEM.68.1.326-334.2002

Zhou, J., Lei, T. 2007 Review and prospects on methodology and affecting factors of soil mi-crobial diversity. Biodiversity Science. 15 (3): 306–311. doi: 10.1360/biodiv.070069