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Impact of land use on microbial communities in Estonian soils

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ORIGINAL PUBLICATIONS

This thesis is based on the following original papers that will be referred to by their Roman numerals in the text.

I Vares A., Lõhmus K., Truu M., Truu J., Tullus H., Kanal A., 2004. Productivity of Black alder (*Alnus glutinosa* (L.) Gaerttn.) on reclaimed oil — shale mining detritus and mineral soils in relation to rhizosphere conditions. Oil Shale, 21, 47–62.

II Nurk K., Truu J., Truu M., Mander Ü., 2005. Microbial characteristics and nitrogen transformation in planted soil filter for domestic wastewater treatment. Journal of Environmental Science and Health, 40, 1201–1214.

III 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 areas. Plant and Soil, 283, 1–10.

IV Truu M., Truu J., Ivask M., 2008. Soil microbiological and biochemical properties for assessing the effect of agricultural management practices in Estonian cultivated soils. European Journal of Soil Biology, 44, 231–237.

V Truu M., Truu J., Heinsoo K., 2008. Changes in soil microbial community under willow coppice: the effect of irrigation with secondary-treated municipal wastewater. Ecological Engineering (in press), doi:10.1016/ j.ecoleng.2008.08.010

Author's contribution

Publication I: The author performed all microbiological analyses (100%), participated in data analyses (15%) and writing the manuscript (20%)
Publication II: The author participated in experiment planning (25%), microbiological analyses (40%) and interpretation of the results (20%).
Publication III: The author participated in experiment planning (20%), performed all microbiological analyses (100%), participated in the data analyses and writing the manuscript (20%).
Publication IV: The author performed sampling (100%), all microbiological and biochemical analyses (100%) and is partly responsible for the data analyses (50%) and writing the manuscript (70%).
Publication V: The author participated in experiment planning (40%), performed sampling (100%) and all microbiological, biochemical and molecular analyses (100%), is partly responsible for the data analyses (50%) and for writing the manuscript (50%).

ABBREVIATIONS

ANAMMOX – anaerobic ammonium oxidation
ATP – adenosine triphosphate
BA – Biolog activity
BD – bacterial species diversity by DGGE
BS – functional diversity
bSh – bacterial diversity by PLFA
bPLFA – bacterial biomass
CV – coefficient of variation
DNA – deoxyribonucleic acid
EDTA – ethylenediamine tetraacetic acid
fPLFA – fungal biomass
HSSFCW – horizontal subsurface flow constructed wetland
mSh – microbial community diversity by PLFA
PCA – principal component analyses
PCR – polymerase chain reaction
PEG – polyethylene glycol
PLFA – phospholipid fatty acid
qCO_2 – metabolic quotient
RNA – ribonucleic acid
SIR – substrate induced respiration
T-RFLP – terminal restriction fragment length polymorphism

1. INTRODUCTION

Soil is a highly heterogeneous environment with a variety of microhabitats characterized by different physicochemical and environmental conditions providing a living place for an intricate network forming a tremendous amount of species. Soil microorganisms have a fundamental role in such complex systems by stabilizing soil particles, performing organic matter decomposition, and mediating nutrient cycling and energy flow (Doran and Zeiss, 2000). The abundance and diversity of soil microbial communities is also huge since one gram of soil can contain up to 10 billion microorganisms from thousands of different species of prokaryotic (bacteria and archaea) and eukaryotic (mostly fungi) divisions (Torsvik and Øvreås 2002). Microbes adapt to the microhabitats forming different consortia interacting with each other and with the other organisms in soil.

Soil health can be defined as a capacity to function as a vital living system within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health (Doran and Zeiss, 2000). Agriculture has been one of the oldest areas of human activity where people have intervened in the natural processes of soil and in this way affecting the soil properties and status. The fast development of technology and concomitant huge waste problem in almost all areas of life, especially in the last decades, emphasizes a need for finding alternative cost-effective biotechnological solutions to the conventional applications (Paranychianakis et al. 2006). The ability of microorganisms to fulfill various important functions in soil enables the successful application of these organisms in different fields of environmental technology. Since microorganisms play a crucial role in degradation processes they are also able to detoxify different pollutants (toxicants), contributing in this way to the soil quality maintenance (Chaudri et al. 2000, Haney et al. 2002, Watanabe and Hamamura 2003). On the other hand, microbial activities are strongly dependent on the nutritional and other chemical and physical conditions of the soil and respond rapidly to the changes in soil properties caused by land management (Böhme et al. 2005, Stark et al. 2007, Wu et al. 2008) or climate (Grierson et al. 1999, Christopher et al. 2008). Due to this fact microorganisms are considered as sensible indicators, when monitoring changes in soil status affected by human activity like agricultural management (Bending et al.2004, Stark et al. 2007, Bossio et al. 2005, Ratcliff et al. 2006, Deurer et al. 2008) or waste treatment (Filip 2002, Castaldi et al. 2004, Tiez et al. 2007). In this context soil quality and health has become one of the major ecological concerns. Nevertheless, the impact of different biotechnological processes like wastewater treatment on soil microbial community (Paranychianakis et al. 2006), as well as the specificity of microbial processes in constructed systems (like constructed wetlands) simulating natural environments, is still poorly studied (Liang et al. 2003, Tiez et al. 2007, Tao et al. 2007). Only very little data is available about soil microbial activity (especially in arable soils) from the Baltic region (Seeman et al. 1998).

2. THE AIM OF THE STUDY

The main aim of this thesis was to evaluate the impact of different land use practices on the microbial communities in Estonian pedogenic — arable soils and anthropomorphic materials — sand filters of horizontal subsurface flow constructed wetland, and materials from opencast oil-shale mining areas (oil-shale mining spoil and Spolic Regosol) with different applications.

The specific aims were:

– to study the impact of agricultural management on the soil microbial biomass, community structure and different activity parameters in Estonian arable soils;
– to study the impact of tree roots and soil properties on the microbial communities in reclaimed abandoned arable soils and anthropomorphic materials from opencast oil-shale mining areas;
– to study the impact of recultivation and pretreated wastewater application into the soil on microbial parameters in arable soils and sand filters of constructed wetland;
– to compare the microbial biomass and activity in disturbed pedogenic soils and anthropomorphic materials with respective microbial parameters in undisturbed, natural pedogenic soils.

3. LITERATURE REVIEW

3.1. Soil as a habitat for microorganisms

There are many different environmental factors, such as temperature (Kern 2003, Paredes et al. 2007), soil moisture (Drenovsky et al. 2004), oxygen conditions (D'Angelo and Reddy 1999, Paredes et al. 2007), pH (Silvan et al. 2003, Fierer and Jackson 2005, Paredes et al. 2007), nutrient concentration and availability (D'Angelo and Reddy 1999, Paredes et al. 2007), affecting different aspects of microbial activity in soil. The size, structure and functioning of the soil microbial community is strongly dependent on soil type (Dilly et al. 2003, Girvan et al. 2003, Ladd et al. 2004, Johns et al. 2004, Väisänen et al. 2007). The heterogeneity in soil even at a small scale (nm) is highly ordered and the resulting spatial clustering of the matrix gives soil its characteristic aggregated structure (Young and Crawford 2004). A broad range in pore sizes permits the coexistence of air and water essential to the biological functioning of soil. The results from geo-statistical methods show that soil organisms also exhibit spatially predictable aggregated patterns over large (hectares) and small (square millimeters) scales (Ettema and Wardle 2002). Analyses of the spatial distribution of bacteria at the microhabitat level showed that more than 80% of the bacteria locate in micropores of stabile soil micro-aggregates (2–20 μm) (Torsvik and Øvreås, 2002). Some functional groups of soil microorganisms, such as indigenous ammonia-oxidizing bacteria, are found to be more strongly (at least 10-fold less extractable) attached to the soil clay particles when compared to the most heterotrophic bacteria (Aakra et al. 2000).

Several authors have found a large consistent soil effect on microbial biomass formation and turnover (Ladd et al. 2004, van Diepiningen et al. 2006). Especially soil clay content but also moisture, and substrate availability form the set of soil conditions which have the characteristic capacity to protect and preserve the microbial community and affect the efficiency of substrate metabolism. According to the authors, clay soils have a greater capacity in such protection compared to sandy loam by providing a sorptive, high surface area environment for closer interactions between microorganisms and their immediate products of decay. These interactions lead to a higher efficiency in the utilization of different substrates (glucose, metabolic products) for biomass production. Soil clay content and soil moisture conditions also determine the leaking process of temporarily available material originating from a semi-closed system formed by soil micro-organisms and their immediate organic products of decay (Ladd et al. 2004).

An important factor is also soil organic carbon content since organic soils (with the upper organic layer containing from 12% to over 20% of organic carbon) in general have a higher nutrient availability and turnover (greater denitrification potential, faster sulfate reduction) compared to mineral soils (D'Angelo and Reddy 1999). The microbial community adapts to the soil-

specific conditions by switching to the respective metabolism type resulting also in different growth rates of these organisms. Bacterial cell division rates in soils with low pH (organic peat soils) are very low from 1cell per 9.3 days to about two divisions per year (Silvan et al. 2003).

Soil can be divided into rhizosphere and bulk soil. Rhizosphere is referred to as the volume of the soil influenced by the roots and the root tissue colonized by micro-organisms, supporting diverse bacteria and fungi that can benefit the growth of plants by fixing nitrogen, enhancing phosphorus solubility and producing phytohormones. Bulk soil is the part of the soil not so closely related to the plant roots where the microbial communities are not as directly affected by root processes. Release of carbon compounds from plant roots into the soil results in greater microbial biomass and activity but at the same time much lower microbial diversity in the rhizosphere as compared to bulk soil (Morgan et al. 2005). Substrate availability is an important regulator of the microbial activity, growth and community structure (Dilly 2001, Cookson et al. 2005). Release of readily available compounds into the soil induces high biomass–specific respiratory activity typical for fresh leaf litter and rhizosphere communities, while organic matter in the bulk soils is largely recalcitrant (Morgan et al. 2005). Studies have shown that glucose stimulates at much higher microbial respiration rate compared to cellulose while the consumption of humic acids and lignin resulted in the lowest respiration rate both in arable and forest soils (Schutter and Dick 2001, Dilly 2004). In addition plant roots enhance the microbial growth by supplying soil microbes with oxygen needed in oxidative processes like nitrification or aerobic respiration (Paredes et al. 2007).

3.2. Soil microbial community

According to the estimates of microbial ecologists the biomass of micro-organisms dominates the soil ecosystem and widespread chemical transformations, as consequences of microbial metabolism, are huge both in magnitude and impact and complex in their details (Rappé and Giovannoni 2003). Microbial diversity describes the complexity and variability at different levels, including the genetic variability within species, the number and relative abundance of species (taxons) and functional groups in communities (Torsvik and Øvreås 2002). Also the functional diversity should be taken into account since different structural groups or species can fulfill similar functions in soil (Johns et al. 2004, van Diepeningen 2006, Paredes et al. 2007). There are a number of estimates available in literature on the size of the whole microbial community (microbial biomass) (Bailey et al. 2002a, Ladd et al. 2004, Bloem et al. 2005) and different groups (functional or structural) of microorganisms in different soils (Lawlor et al. 2000, Nakatsu et al. 2000, Pennanen et al. 2001, Torsvik and Øvreås 2002). These estimates can vary greatly in some cases depending on the methods used (Bailey et al. 2002a, Torsvik and Øvreås 2002).

An estimation based on the results obtained with the plate count method reported 10^{11}–10^{15} bacteria and 10^{5}–10^{6} fungal colony-forming units in one gram of terrestrial soil (Lawlor et al. 2000).

Prokaryotic organisms such as bacteria and archaea are essential soil components decomposing plant and animal residues thus actively participating in nutrient cycling and energy flow (Friedrich et al. 2001, Silvan et al. 2003, Truu et al. 2005). The estimated overall number of prokaryotic microorganisms is consistent (about 2.6×10^{29} cells) in terrestrial soils and its average turnover time is calculated to be 2.5 years (Whitman et al. 1998).

The prokaryotic microorganisms actively participate in the carbon cycle in soil — they decompose organic compounds (sugars, organic acids etc.), but also produce different organic compounds like organic acids and methane in anaerobic conditions.

Different transformation mechanisms are used by soil bacteria to convert organic nitrogen (amino acids, amino sugars, urea and uric acid etc.) into ammonium, the preferred nutrient form of nitrogen for plants and autotrophic bacteria. Under aerobic conditions ammonium is oxidized to nitrate by two different groups: ammonium and nitrite oxidizing bacteria. Denitrifying bacteria reduce nitrate to dinitrogen gas closing thus the global nitrogen cycle. The excess nitrate is removed from the soil during denitrification process prior to its movement to the ground or surface water. On the other hand, N_2O — important greenhouse gas consuming stratospheric ozone is produced during denitrification.

In 1995 Mulder and coauthors discovered a novel process called ANAMMOX (anaerobic ammonium oxidation) from a denitrifying bed reactor. In this process under anaerobic conditions and low organic carbon concentrations autotrophic ANAMMOX bacteria are able to use nitrite (and ammonium) as an electron acceptor and bicarbonate as a carbon source to convert ammonium and nitrite to nitrogen gas. ANAMMOX bacteria (*Brocadia anammoxidans*, *Kuenenia stuttgartiensis*, *Scalindua sp*. etc.) are slow-growing and very sensitive to oxygen but also nitrite concentrations, and therefore dependent on the other functional groups of bacteria (Paredes et al. 2007).

The role of prokaryotes is important in soil phosphorous and sulfur cycling. Reduced inorganic sulfur compounds are exclusively reduced by a phylogenetically diverse group of prokaryotes — from domain *Acrhaea* by members of the order *Sulfolobales* and from domain *Bacteria* by aerobic lithotrophs and anaerobic phototrophs (Friedrich et al. 2001). The mineralization of phosphorus in soil is mediated by a microbially and plant produced group of enzymes — phosphatases that hydrolyze esters and anhydrides of phosphoric acid. Microbial phosphatases dominate in soil (Schinner et al. 1996) and bacteria contribute to this activity (Singh et al. 2006).

Albeit the fungi fulfill a range of important ecological functions in soil, particularly associated with nutrient cycling processes because of their ability to degrade complex substrates of plant origin (lignin, cellulose, hemicellulose etc.)

representing up to 90% of the net primary production of terrestrial ecosystem (Priha et al. 2001, Follett et al. 2007), the understanding of the fungal diversity and functioning in the soil ecosystem is much poorer than compared to the bacterial community. An estimated 1, 500, 000 species of fungi exist in the world but because most of the current fungal taxonomy is based on fungal sexual states (mushrooms, truffles), the identification of the below-ground vegetative structures remains problematic (Kirk et al. 2004). Fungi with longer life-spans are found to predominate over bacteria in low pH organic peat soils prolonging in this the way the microbial nutrient immobilization period compared to mineral soils with high pH (Silvan et al. 2003). In addition to decomposition ability, soil fungi also have an important role in the succession of plant growth by forming symbiotic associations with plant roots known as mycorrhiza. The estimated number of fungal species participating in symbiotic relationships with higher plants is 7,000–10,000 from phyla *Basidomycota*, *Ascomycota* and *Zygomycota* (Tedersoo 2007). The primary function of the fungal mycelium is the absorption of nutrients (mostly mineral forms of nitrogen, phosphorus and micronutrients) from the soil and their transport to the host. Evidence has been found that ectomycorrhizal richness can enhance plant phosphorus uptake under certain conditions (Baxter and Dighton 2005) but the studies also have shown that fungal diversity decreases in the rhizosphere along with a plant age effect (Anderson and Cairney, 2004).

Microbial community structure and functioning is dependent on land use and soil management (Filip 2002, Blagodatskii et al. 2008). Broad-scale analyses found, by using microbial community DNA, that the genome size of un-perturbed organic soils equals the size of 6000–10000 *Escerichia coli* genomes and 350–1500 genomes in arable or heavy metal-polluted soils (Torsvik et al. 1998, Øvreås 2000). Agriculture is one of the oldest and most important areas of human activity affecting soil physical-chemical and microbiological (functional and structural) properties (Girvan et al. 2003, Bloem et al. 2005, van Diepiningen et al. 2006). Studies have shown that changes in land use can also affect soil microbial community, for example a conversion from native forest to plantation, is accompanied with changes in fungal community structure (Bastias et al. 2007), while some changes such as abandonment of arable land (changes in plant species composition and diversity) may not be reflected in microbial biomass and activity after a short period (2 years) of time (Malý et al. 2000).

3.3. Microbiological parameters in evaluation of soil status

3.3.1. Microbial biomass and activities

The microbial parameters used to evaluate soil status should meet five main criteria: they have to be sensitive enough to the variations in soil management

and correlate well with beneficial soil functions, be useful for elucidating ecosystem processes, but also comprehensible and useful for land managers, and in addition easy and inexpensive to measure (Doran and Zeiss 2000).

Microbial biomass carbon was found to be one of the reliable parameters indicating the amount of organic carbon readily utilizable by heterotrophic microorganisms as well as useful for predicting different heterotrophic processes (O_2 consumption, denitrification, sulphate reduction, methanogenesis) in several soils from natural (forest, arable) (Filip 2002, Dilly 2004) and artificial environments like constructed wetlands (D'Angelo and Reddy 1999, Tiez et al. 2007). There are a number of different methods (substrate induced respiration, fumigation-extraction of microbial C, N and P, ATP measurements, etc.) available in literature enabling the assessment of this parameter in different soils (Schinner et al. 1996, Margesin and Schinner 2005, Tiez et al. 2007).

Soil respiration reflects the degradation of organic matter where the formation of CO_2 is the last step of carbon mineralization and considered as a measure of the total soil biological activity (Schinner et al. 1996, Filip 2002). Any disturbances such as the addition of organic matter or changes in labile (sugars, organic acids etc.) and stabile (humic acids, fulvic acids, humin etc.) fractions of organic matter can be observed as a change in the soil respiration activity as has been reported by Nguyen (2000) in wetlands and by Dilly (2004) in forest and agricultural soils.

The microbial community potential to fulfill a range of important functions, like carbon and nitrogen mineralization and uptake, phosphorous and sulfur transformations and others, under certain conditions can give valuable information about different anthropogenic influences to the soil quality (Filip 2002). Short term (from 1 h to several weeks) laboratory experiments under controlled conditions based on measurements of specific enzyme (urease, protease, different phosphatases, dehydrogenase etc.) or process activities (like N-mineralization, potential nitrification etc.) are applied for this purpose (Kang et al. 1998, Sparling et al. 2004, Zhou 2005). This approach has been used most frequently in studies of agricultural management impact on soil microbial communities due to the ecological and economical concern caused by long-term and continuous anthropogenic pressure and concomitant reduction of arable soil quality (Dilly et al. 2003, Senwo et al. 2007). The enzymatic (activity) approach has been successfully applied also in studies of decomposition processes in wetland sediments (Kang et al. 1998), forest (Billings and Ziegler 2008) and grassland soils (Tscherko et al. 2004), assessing the influence of heavy metals on soil microbial activity (Stuczynski et al. 2007), and has also been introduced into constructed wetland studies (Zhou 2005, Sundberg et al. 2007)

3.3.2. Molecular methods in soil microbial ecology

It is widely known that less than 1% of microorganisms from the natural environment are culturable making them rather complicated to separate, determinate, and describe (Torsvik and Øvreås 2002). Methods based on the extraction and further analyses of specific biomolecules, like phospholipid fatty acids (PLFA) — the essential cell membrane components (Bailey et al. 2002a, Ebersberger et al. 2004, Ratcliff et al. 2006) and nucleic acids (DNA, RNA) inherent to soil microbes have been developed to overcome the cultivation problem (Muyzer et al. 1993, Tindall et al. 2000, Kent and Triplett 2002, Osborn and Smith 2005, Tedersoo 2007). Řezanka with coworkers (1991) identified, by capillary gas chromatography-mass spectrometry, more than 60 long-chain fatty acids in soil oligotrophic bacteria. The individual fatty acid contents can vary largely between bacterial species (Temina et al. 2007). PLFA analyses enables the measurement of total microbial biomass and provides the opportunity to differentiate between soil bacterial and fungal biomass since one phospholipid fatty acid (18:2ω6) was found to be in good agreement with ergosterol concentrations and considered as a biomarker for fungi (Frostegård and Bååth 1996, Bailey et al. 2002b, Wilkinson et al. 2002). In addition some conclusions about bacterial diversity can be made from the obtained data since several fatty acids like i15:0, a15:0, i16:0, i17:0, a17:0 are found to be specific for Gram-positive and 16:1ω7 and 18:1ω7, cy17:0, cy19:0, 16:1a, 16:1b for Gram-negative bacteria (Summit et al. 2000, Billings and Ziegler 2008). Specific biomarkers 10me18:0 (Zhang et al. 2007, Billing and Ziegler 2008) and 10Me 16:0 (Zhang et al. 2007) were found for *Actinomycetes* from the group of Gram-positive bacteria. PFLA analyses have been applied in studies of management impact on fungal:bacterial biomass ratio in bulk soil (Bardgett et al. 1996) and rhizosphere microbial communities of grassland soils (Tsherco et al. 2004), assessing microbial biomass and community dynamics in hot hydrothermally influenced sediments (Summit et al. 2000) and evaluating management impact on microbial communities in forest (Ratcliff et al. 2006, Billings and Ziegler 2008) and agricultural soils (Bossio et al. 2005, Cookson et al. 2005, Zhang et al. 2007).

The analyses of soil microbial community DNA provides information on structural diversity (DGGE, T-RFLP) and permits the evaluation of the presence of certain functional genes in the studied environment, while information can be obtained from RNA analyses about metabolic activity and gene expression (Osborn and Smith 2005). The application of these techniques gave a more than 200 times higher diversity of total soil bacterial community when compared to the results obtained using cultivation based methods from the natural environments (more than 10000 different bacterial types) and environments with an anthropogenic influence (Torsvik et al. 1998). In addition, several important conclusions were reached, like soil type is the key factor determining bacterial community composition in arable soils (Girvan et al. 2003) and the community

structure of *Eubacteria*, *Actinomycetes*, ammonia-oxidizers and *Archaea* is different in organically and traditionally managed soils (Kuffner et al. 2004) but also others, due to the application of these methods. The diversity of ammonium-oxidizing bacteria in different soils has been one of the well studied topics in microbial ecology because of the importance of these microorganisms in nitrogen cycling and especially due to the availability of nucleic acids based methods (Kowalchuk et al. 1998, Bruns et al. 1999, Bothe et al. 2000, Aakra et al. 2001, Nicolaisen and Ramsing 2002). In recent years these nucleic acids based methods have become widely used in different studies of soil microbial ecology (Truu et al. 2005, Shegers et al. 2005, Stark et al. 2007).

In addition, DNA and RNA based methods have the potential to give valuable information about fungal communities in soil. They have been successfully applied in studies of total and active fungal communities in the bulk soil of different forest types (Bastias et al. 2007), in mycorrhizal fungi diversity and community structure assessments (Hempel et al. 2007, Tedersoo 2007) and in studies of different functional groups like cellulolytic fungi for example (Edwards et al. 2008).

4. MATERIAL AND METHODS

4.1. Sampling

Two different groups of soils are studied in the present thesis. The first, pedogenic soil group consists of abandoned, agriculturally managed, reclaimed, and reclaimed and wastewater treated arable soils. They are also considered as disturbed soils in the current work because of the relatively strong influence of human activity. The reclaimed arable soil group consists of former agricultural soils planted with different tree species (*Salix* sp., *Alnus incana*, *A. glutinosa*, *Picea abies*, *Betula pendula* and *Pinus sylvestris*). The ages of the plantations varied from one growing season to 47 years.

According to the definition given by Nachtergaele (2004) the anthropomorphic materials (AM) derive from human activity and have not been subject to the sufficiently long period of soil formation to acquire distinct signs of soil pedogenic alteration. In the current study AM groups were defined as follows: 1) abandoned mining AM group — soils (oil-shale mining spoils and Spolic Anthrosols) derived from oil-shale mining detritus in opencast areas without tree plantations, 2) reclaimed AM group — the soils (oil-shale mining spoils and Spolic Anthrosols) from opencast oil-shale mining areas reclaimed by planting trees, and 3) sand filters (SF) of horizontal subsurface flow constructed wetland (HSSFCW). Reclaimed mining AMs were planted with different tree species (*Alnus incana*, *A. glutinosa*, *Picea abies*, *Betula pendula* and *Pinus sylvestris*) and the ages of plantations ranged from one to 28 years.

Soils from natural wetlands, meadows and wooded meadows are considered as undisturbed (minimal human disturbance) pedogenic soils and used for comparison in the current study.

Details of sampling time, number of samples and measured microbiological parameters for each group of soils is given in Appendix Table 1.

Sampling was performed in the case of all agriculturally managed and natural soils as described in Paper IV.

The sampling procedure for the reclaimed wastewater treated soils is described in Paper V. The samples from this study were included into the data analyses of the soil groups as follows: 1) samples from the plots without trees (plot no 1 in 2003 and plot no 10 in 2003 and 2005) and samples taken from all the plots covered with willow trees not yet influenced by tree roots in 2003 (between tree rows) referred to as abandoned arable soils; 2) samples taken from the tree rows of all planted plots in 2003 and all samples (except for 20 — 30 cm layer) taken from the planted control plots in 2005 referred to as reclaimed arable soils; 3) samples from the planted and wastewater treated plots in 2005 referred to as reclaimed and wastewater treated arable soils; and 4) samples from the plots without trees but treated with wastewater referred to as wastewater-treated arable soils. Only 0 — 10 cm and 10 — 20 cm soil layers were included in the analyses.

The sampling of the rest of the reclaimed arable soils (except for the pot experiment) and mining AMs from abandoned oil-shale mining quarries (abandoned and reclaimed) are given in Papers I and II.

In the case of the pot experiment tree seedlings (spruce and/or birch) were grown for one growing season in pots under field conditions. The plants and all roots were removed from the soil after the leaves had fallen. The soil was sieved (mesh size 2 mm) and microbiological, biochemical and chemical analyses were performed.

4.2. Microbial biomass

The substrate induced respiration (SIR) method by Izermeyer technique as described in paper II, III, IV and V was used to measure metabolically active microbial biomass in all soils.

4.2.1. PLFA analyses

The composition of phospholipid fatty acids (PLFA) was analyzed according to the procedure described by Bardgett et al. (1996) in order to measure total microbial biomass-C. Single-phase chloromethanol-citrate buffer mixtures (Bligh and Dyer 1959) for the lipids extraction and SI-columns (Varian, Harbour City, CA, USA) for the following lipid fractionation were used. Methyl-nonadecanoate was added to the polar lipid fraction and phospholipids were then converted to the fatty-acid methyl esters by mild alkaline methanolysis. Fatty acid methyl esters were analyzed by capillary gas chromatography (Perkin-Elmer Autosystem XL, Norwalk, CT, USA, fitted with a 50 m capillary column [HP-5, Agilent, Palo Alto, CA, USA] and a flame ionization detector). SUPELCO quantitative standards (Sigma-Aldrich, Taufkirchen, Germany) were used for the detection of phospholipid fatty acids from methyl esters. The chemical structure of the methyl esters standards was determined by mass spectrometry. All samples were analyzed using a set of 25 fatty acids. Bacterial biomass (bPLFA) was estimated from the summed concentration of 18 bacterial PLFA: 14:0, i15:0, a15:0, 15:0, i16:0, 16:0, 16:1ω7, i17:0, cy17:0, 17:0, cy15:0, 18:1ω7, 18:1ω9, 18:0, cy19:0, 20:5, 20:0, 22:0, 24:0 (Řezanka et al. 1991, Bardgett et al. 1996, Summit et al. 2000, Temina et al. 2007) and fungal biomass (fPLFA) was calculated from the concentration of the biomarker 18:2ω6 (Frostegård and Bååth 1996). The prefixes a and i in the names indicate anteiso- and iso- branching respectively, and cy cyclopropyl fatty acids, while the numbers symbolize the total numbers of carbon atoms and the numbers of double bonds, followed by the position of the double bond (ω) from the methyl end of the molecule (Ebersberger et al. 2004). Shannon diversity indexes were

calculated from the PLFA data as the measures of total microbial (mSh) and bacterial community (bSh) diversities.

4.3. Enzymatic and process activities

All activity measurements were performed as described in Papers I, II, III, IV and V.

BiologEco microplates were used in order to determine summed activities (Biolog activity — BA) of soil microbial communities (Paper I, II, III and V) and also the functional diversity (BS) — Shannon diversity index was calculated from obtained data. In all cases color development on microplates after 48 h was measured except for sand filters of constructed wetlands where the microbial growth was very low. Shannon diversity indexes for the sand filters were calculated from 72 h growth results.

The metabolic quotient (qCO_2) was calculated as a ratio of soil microbial respiration (basal respiration) and soil microbial biomass (SIR) showing the amount of CO_2-C produced per unit of microbial biomass of carbon (Anderson and Domsch 1993).

4.4. DGGE analyses

The structural (species) diversity of bacterial communities in agriculturally managed arable soils was detected using PCR-DGGE analyses. DNA was isolated from samples with the Fast DNA® Spin Kit for Soil (Bio101) and purified with phenol and PEG treatment according to Pennanen et al. (2001). PCR was performed using bacteria specific F984GC and R1378-1401 primers (Heuer et al. 1997). PCR products were examined by agarose gel electrophoresis (1% agaros, 0.5 × Tris-boreate- EDTA).

For DGGE analyses, polyacrylamide gels (8% polyacrylamide, 16 × 16 cm, 1.5 mm thick) were run at a 1×TAE buffer (40 mM Tris-acetate, 1 mM EDTA [pH 8.0]). The denaturant gradient ranged from 35 to 55% denaturant (100% denaturant contained 7M urea and 40% formamide in the 1×TAE buffer). Gels were run on DCode Detection System (Bio-Rad) at 60 ºC for 16 h at 90 V. For the DNA staining the gels were kept for 40 min in citric acid (10% v/v), washed 3 times with bidistilled water (for 3–5 min each time) and then stained for 30 minutes with the mixture of 0.1% $AgNO_3$ (100 ml) and 37% formaldehyde (0.3 ml), briefly washed with bidistilled water and treated 2×200 ml with the cool developer mixture (0.3% Na_2CO_3 [80 µl] , 47% formaldehyde [0.6 ml] and 0.314% N_2SO_3 [50 ml]). The color development reaction was stopped with 400 ml of 10% citric acid (shaken for 10 minutes) and finally the gel was washed with 400 ml of bidistilled water (20 minutes).

The stained gels were scanned and the digitalized images were processed using GelCompar Version 4.0 software.

The bacterial species diversity index (Shannon diversity index — BD) was calculated from all DGGE gels used in the analyses of the current work.

4.5. Statistical analyses

The Spearman rank correlation coefficient was used to relate soil microbiological variables to chemical parameters. This analyses was not applied for wastewater treated arable soils, soils from meadows (natural pedogenic soils) and abandoned mining AMs because of the small number of samples analyzed (n=4, n=4 and n=3 respectively). The data set of the soil microbiological variables was analyzed using principal component analysis (PCA) based on correlation matrix. Prior PCA analysis values of microbiological variables were log-transformed. The data set for PCA did not contain natural soils and sand filter groups since not all microbiological parameters were measured in these soils. The Mann-Whitney test was applied to compare microbiological variable values and the t-test was applied to compare Shannon diversity indexes between groups.

5. RESULTS AND DISCUSSION

5.1. Microbial biomass and activity in soils under different application

The values of measured chemical and microbiological parameters for studied soil groups are given in Appendix (Table 2 and Table 3 respectively). When looking at all the soils together, the metabolically active microbial biomass, microbial respiration, potential nitrification and alkaline phosphatase activity were the most variable microbiological parameters (CV 105%, 107%, 126% and 125% respectively) while the metabolic quotient (qCO_2), N-mineralization, acid phosphatase and summed activities of the microbial communities did not vary as much (CV 58.4%, 54.1%, 36.6% and 44.8% respectively). The least variable was the functional diversity (FD) of the microbial communities (CV 9.06%). Statistical analyses revealed that the most variable soil chemical parameters for all the studied soils were soil total nitrogen and organic matter content (CV 113% and 101% respectively), but at the same time these two parameters had strong and statistically significant correlations with metabolically active microbial biomass, microbial biomass C/N, respiration and alkaline phosphatase activities (Appendix Table 4), reflecting their importance to the microbial community in soil. Soil pH, phosphorus and potassium status were related only to the potential nitrification activity. The analyses of each studied group separately revealed differences between groups reflected by correlations between chemical and microbiological parameters (Appendix Tables 5, 6, 7, 8, 9, 10, 11 and 12). These differences suggest that there can be different mechanisms due to the specific conditions such as the amount and composition (the ratio between labile and stable fractions) of soil organic matter, type and form of nutrient supply (in organic or inorganic form of N and P) (Billings and Ziegler 2008) involved in the nutrient cycling in soils with different applications.

Based on microbial biomass and respiration data for all studied soils, natural (undisturbed) soils could be clearly distinguished from disturbed — arable soils and the AM group (Figure 1a) with one exception — AMs from reclaimed mining areas that exposed high microbial biomass and activity in some cases (high variability within the group) comparable to the natural soils. The arable soils with different active land use (agriculture, reclamation, wastewater treatment) can be distinguished, according to the biomass and respiration data, from the abandoned arable soils and AMs (Figure 1b) that had the smallest and not very active microbial biomass. In the case of the pedogenic soil group the qCO_2 was statistically different (Mann-Whitney test, $P<0.05$) in natural and arable soils (0.23 ± 0.09, $n=111$ and 0.28 ± 0.11, $n=29$ respectively). These values are slightly higher from values (between 0.1 and 0.2) for natural biotopes (forest and meadows) as reported by Blagodatskaya and coauthors (1996). Significantly higher values of this parameter in the AM group (Mann-Whitney test, $P< 0.001$) compared to pedogenic soils, particularly in sand filter and abandoned

mining AMs indicate the instability of microbial communities in these environments (Ananyeva et al. 2002, Yan et al. 2003, Rietz and Haynes 2003).

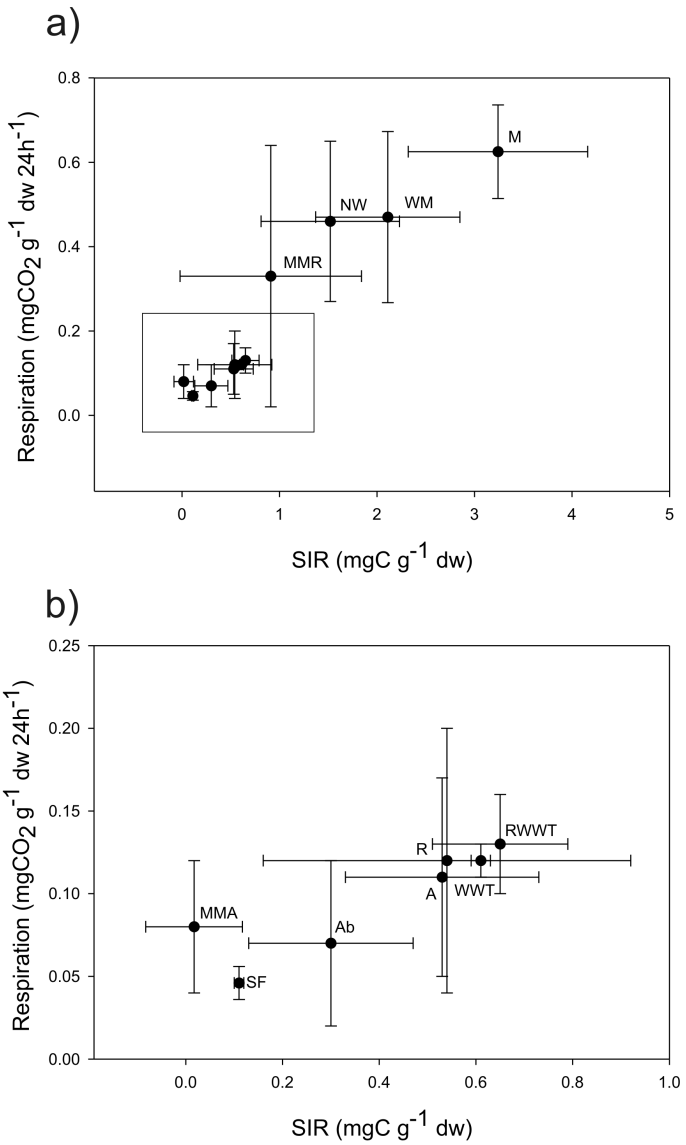

Figure 1. Plot of microbial active biomass and respiration activity for studied soils groups. Shown are mean values and standard deviations. Abbreviations: M — meadows, WM — wooded meadows, NW — natural wetlands, MMR — reclaimed mining AMs, MMA — abandoned mining AMs, A — agricultural soils, Ab — abandoned arable soils, R — reclaimed arable soils, WWT — wastewater treated arable soils, RWWT — reclaimed wastewater treated arable soils, R — reclaimed arable soils, SF — sand filters.

In multivariate analysis, abandoned mining AMs are grouped separately from the rest of the soils (Fig. 2a). In addition to significantly lower biomass, respiration and potential nitrification values in these soils, they are also characterized by low nitrogen mineralization and acidic phosphatase activity (Figure 2b). Arable soils differ from each other mostly by biomass, respiration, potential nitrification and alkaline phosphatase activity values. Within this group these parameters are the lowest in abandoned and reclaimed oils.

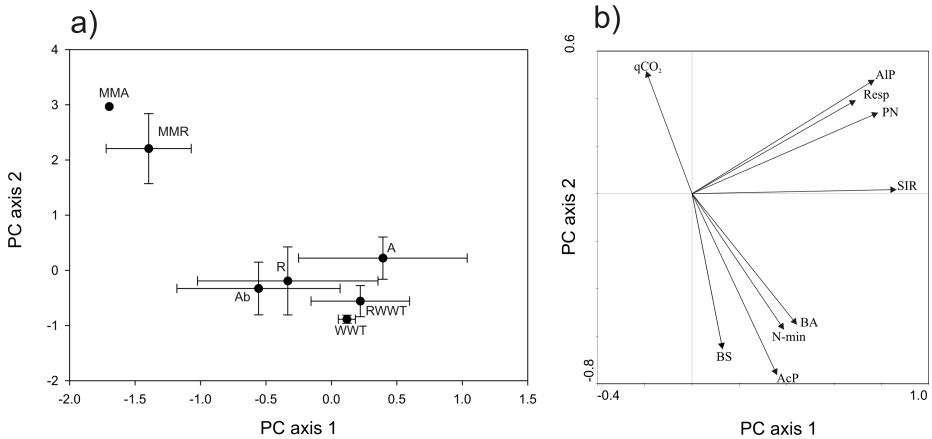

Figure 2. Principal component analysis based on correlation matrix of soil microbiological parameters. a) Plot of groups on plane of two first principal component axes. Shown are mean values and standard deviations for group member scores. b) Correlations of soil microbiological parameters with two first principal component axes. Two first principal component axes describe 33.0% and 26.8% of total variance, respectively. Abbreviations are the same as on Figure 1.

5.2. Response of soil microbial biomass and activity to agricultural management (Paper IV)

Soil total nitrogen and organic matter content but also soil pH are important factors affecting both active (SIR) and total microbial biomass (PLFA) but also fungal (fPLFA) and bacterial (bPLFA) fractions and their activity in agriculturally managed soils (Appendix Table 5). Active microbial biomass is strongly correlated with total microbial biomass ($R=0.76$, $P<0.0001$). The fraction of active microbial biomass was between 25.0 and 72.2% in studied agriculturally managed soils. According to Stenström and coauthors (2001), the fraction of growing and non-growing (dormant) microbial biomass can be from 5 to 100% depending on substrate availability, while the transition between dormant and active states is ruled by community-level control. These two biomass fractions gave slightly different patterns of correlations in measured

activity and also structural parameters (Appendix Table 13) suggesting that total biomass reflects better the potential of the microbial community to fulfill important functions in agricultural soils. Both bacteria and fungi contribute to this activity as the data analyses revealed. Surprisingly, the alkaline phosphatase activity had very strong correlations with both groups of microbes while acid phosphatase activity was correlated only with bacterial biomass (Discussion in Paper V).

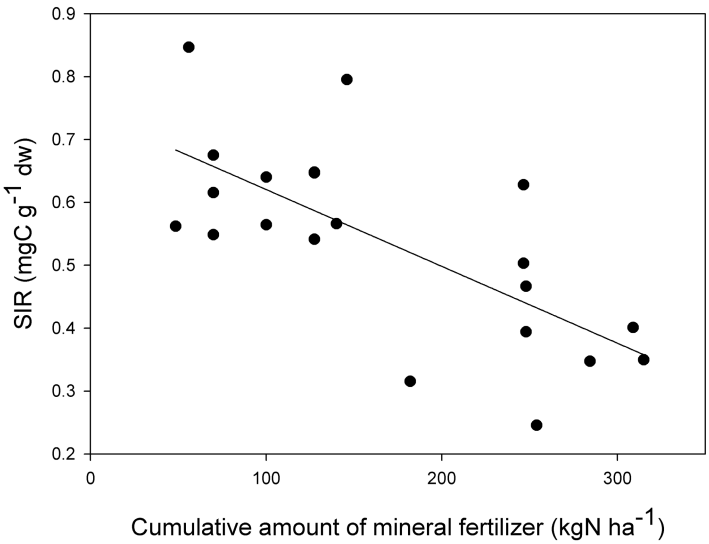

Figure 3. Relationship between cumulative amount of mineral nitrogen applied during last three years and active microbial biomass in arable soils.

Agricultural management practice includes a range of individual activities that reflect on soil microbiological properties (Paper IV). For example, repeated mineral nitrogen application leads to the decrease of soil active microbial biomass (Figure 3). The negative effect of long-term mineral fertilizer application on microbial biomass is reported also by other authors (Sarathchandra et al. 2001, Bittman et al. 2005). An observed decrease can occur due to shifts in organic matter composition (Discussion in Paper IV) and concurrent structural changes in the soil microbial community. Gram-positive bacteria (particularly actinomycetes) perform active soil organic matter transformation in nitrogen limiting conditions and responded negatively to the mineral N-fertilization in forest soils (Billings and Ziegler 2008). Seghers with coauthors (2005) found that the community structure of methanothrophs differs, and the abundance of these organisms is higher, in soils receiving organic fertilizers compared to those receiving mineral fertilizers.

Potassium is an important nutrient for crop production and is added into the soil by fertilization in the case of agricultural management. Municipal wastewater can contain also considerable amounts of potassium introduced into the soil during the process of wastewater application (Schonborn et al. 2001). In our study, the soil potassium concentration was also increased after two years of wastewater application to the willow plantation growing on abandoned arable land (Paper V). Statistical analyses revealed that potassium was positively related to the microbial activity parameters connected to nitrogen cycling (potential nitrification and N-mineralization) and basal respiration in abandoned (Appendix Table 6) and reclaimed (Appendix Table 7) arable soils. Correlations between potassium and microbial activity parameters were found in soils where the addition of potassium was stopped. In soils where potassium was still added (agriculturally managed and wastewater receiving arable soils) these kinds of relationships were not detected. Potassium is an important cofactor for several bacterial enzymes including some of those involved in protein synthesis (Madigan et al. 2000). In addition, the intracellular K^+ pool is used by some groups of prokaryotes (Gram-negative heterotrophic bacteria) to overcome several different (pH, osmotic, oxidative etc.) stresses (Oktyabrsky and Smirnova 1993, Masip et al. 2006). Changes in land use can cause changes in soil conditions (including lower potassium concentration) that forces the microbial community to adapt to the new situation which can lead to the shifts in microbial community structure. This is also reflected by a positive correlation between functional diversity and potassium concentration in reclaimed arable soils (Appendix Table 7).

The structural diversity of bacterial communities in the agriculturally managed soil group did not differ from the diversities measured in the other (reclaimed, abandoned and wastewater treated) arable soil groups (t-test, $P > 0.05$).

5.3. Response of soil microbial biomass and activity to land abandonment

Two different groups — previously actively agriculturally managed soils and AMs from oil-shale mining areas are considered in this section. Results from data analysis showed that both groups of abandoned soils had low microbial biomass and respiration activity (Appendix Table 3). The basics for the microbial processes differ between these groups. The AMs have not been subject to pedogenetic alterations for a sufficiently long period and are characterized by low microbial colonization and biomass in the early soil development stage (Lorenz and Kandeler 2005). High metabolic quotient also reflects stress conditions probably due to the N deficiency (0.03±0.003%, n=4) and instability of developing microbial communities in this environment (Yuan et al. 2007).

The abandoned arable soils have gone through long-term evolutionary processes (pedogeneses) and have acquired the structure and properties providing conditions for better stabilized microbial communities reflected by their lower qCO_2. Very few and not strong correlations between measured chemical and microbiological parameters were found in this group of soils (Appendix Table 6). No correlations were found between chemical parameters and microbial biomass (SIR), while respiration activity was related only to soil potassium concentration in this group of soils.

5.4. Response of soil microbial biomass and activity to reclamation by planting trees (Paper I, II and V)

Reclaimed arable soils and abandoned oil-shale mining quarries are the two groups that have been considered in this section. An analysis of all reclaimed soils together reveals a relationship between active soil microbial biomass and the age of the trees (Figure 4).

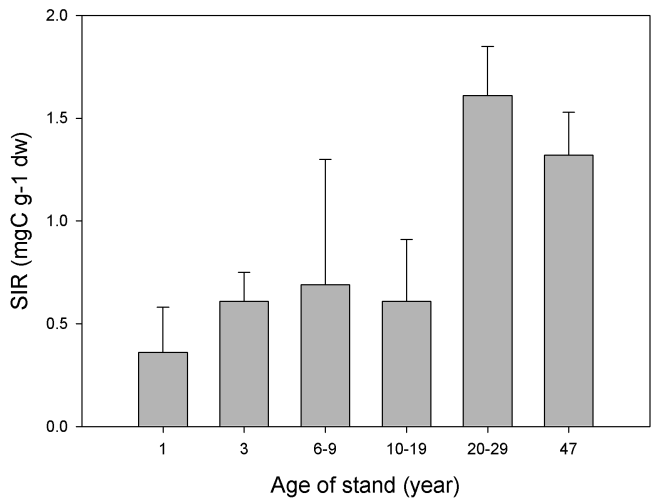

Figure 4. Relationship between stand age and active microbial biomass. Mean values and standard deviations are shown.

The fast increase of active microbial biomass in bulk soil in the first (1–9) years of the tree growth is obvious. The statistical analyses showed differences in correlations between microbial biomass and measured activity parameters, with soil chemical parameters (Appendix Tables 7 and 8). This dissimilitude can be partly caused by different soil conditions and interactions between plant roots and microorganisms in these soils (Results and Discussion in Paper I, II and V).

5.5. Response of soil microbial biomass and activity to wastewater treatment (Paper III and V)

Wastewater treatment had a positive effect on the soil microbial community — the microbial biomass in both soil groups (only wastewater treated, and wastewater treated reclaimed soils) was twice as high as the respective value for abandoned arable soils (Appendix II Table 3) but still much smaller than in natural soils. The microbial respiration activity in wastewater treated arable soils was comparable to that of the agriculturally managed and reclaimed arable soils. Low qCO_2 values indicate stable and effectively functioning microbial communities since high Biolog activity and acid phosphatase activity values were measured for these groups. A high N-mineralization activity in these soils and low nitrification potential was measured in both groups probably because of the composition of the applied wastewater. A large part of the nitrogen in wastewater can be in organic form and has to be mineralized before use by the soil microorganisms.

The structural diversity (DGGE) of the bacterial community was significantly higher in wastewater treated than non-treated arable or natural soils (t-test, $P<0.05$). Also functional diversity differed between wastewater treated and non-treated arable soils (t-test, $P<0.05$). The measured data suggests that short-term pretreated municipal wastewater application had a weak effect on microbial properties in the arable soils (Paper V).

Constructed wetlands are artificial environments where the microbial community depends on, in addition to environmental factors (Scholz and Lee 2005), also the nutrient supply introduced by wastewater (Tanner et al. 2002, Shackle et al. 2000), particle size of filter material (Nguyen 2001), water table in filter body (Scholz and Lee 2005), and other conditions during the operational period. The results and discussion about microbiological parameters in the municipal wastewater treating HSSFCW sand filter is given in Paper V.

The highest qCO_2 value (almost three times higher compared to natural wetlands) among the studied soil groups indicated inefficient microbial communities probably because of increased need for energy in unstable conditions or unfavorable compounds contained in the wastewater. The microbial biomass was small and respiration activity was low in the studied soil filters, while the nitrification potential of the community was comparable to the reclaimed arable soils and exceeded almost 4 times of that measured in arable soils treated with wastewater. The functional diversity was lower in sand filters compared to the wastewater treated arable soils (t-test, $P<0.05$) and all arable soils (2.78 ± 0.17, $n= 111$, t-test, $P<0.001$) with different applications, but did not differ from mining AMs (t-test, $P>0.05$). Potential nitrification activity was comparable to the reclaimed arable soils but still remained almost 5 times lower from the respective value for the agriculturally managed soil group.

Duncan and Groffman (1994) concluded from the results of their comparative study that constructed wetlands have active microbial communities that

facilitate nutrient cycling similar to natural wetlands. In the current study the microbial biomass was much (13.8 times) smaller and the respiration activity was considerably lower (9 times) in sand filters of the horizontal subsurface flow constructed wetland compared to the soils from natural wetlands. Different chemical parameters affected microbial biomass and activity in natural and artificial environments (Appendix II Tables 9 and 10).

In natural wetlands, receiving additional potassium in the course of flooding especially by sea but also by river water, potassium was negatively related to the active microbial biomass (Appendix Table 11). Together with potassium, also some unfavorable compounds (salts) for soil microbes can be carried into wetland soils during flooding. Stress conditions in these soils are reflected also by a smaller microbial biomass and higher mean qCO_2 value compared to the values measured for other natural soils (Mann-Whitney test, $P<0.01$ and $P<0.05$ respectively). Yuan with coauthors (2007) also found that higher salinity results in a smaller, more stressed and less metabolically efficient microbial community.

6. CONCLUSIONS

Based on the results of this study it could be concluded that land use (soil application) considerably affected soil microbiological and biochemical properties. The response of the microbial community to soil management was dependent on soil type and other site specific conditions like the amount and form of nutrients in the soil. In pedogenic soils microbial communities had a bigger capacity to sustain its functions in changing conditions compared to the young communities in AMs. Arable soils (disturbed pedogenic soils) had a smaller and less active microbial biomass compared to natural soils. The sand filters of HSSFCW and abandoned mining soils from the anthropomorphic material group were characterized by the smallest microbial biomass among the studied soil groups while anthropomorphic materials from reclaimed mining areas presented intermediate (transitional) microbial biomass and activity values.

Arable soils with different applications had well stabilized, effectively functioning microbial communities that could be separated from each other by their biomass and different activity parameters like respiration, potential nitrification, and alkaline phosphatase activity.

Agricultural management practices include a range of different activities that specifically affect microbial biomass and its activity in arable soils. Soils, in which the principal rules of organic management have been followed, are characterized by a bigger microbial biomass and higher activities compared to conventionally managed soils. Organic fertilization and legume–based crop rotation increased microbial biomass and elevated its activity while long-term mineral N fertilization resulted in reduced microbial biomass in soil. Both fungal and bacterial communities contribute to the microbial activity in agricultural soils.

Soil microbes adapt to the conditions (artificial introduction of nutrients, destruction of soil structure by cultivation etc.) created by particular agricultural management type. Changes in land use force soil microbes to restructure their communities according to the new conditions and these changes can be monitored by shifts in microbiological parameters.

The abandoned agricultural soils had the smallest and least active microbial biomass in the arable soil group. The abandoned mining AMs had even lower microbial characteristics. Reclamation of these soils by planting trees increased microbial biomass and elevated its activity. The rapid increase in microbial biomass in bulk soil was observable in the first years of tree growth. The formation of microbial community functional structure was mostly dependent on soil characteristics but also on tree species. The establishment of willow plantations on abandoned agricultural soils resulted in higher bacterial diversity and increased similarities between microbial communities in the studied soils.

In the case of black and grey alder trees, the roots created favorable conditions for microbes in the soil-root interface where the functional diversity

and activity were higher when compared to the bulk soil. These differences were more pronounced, due to the plant support, in less favorable soil conditions like young stands on oil-shale mining spoil. Rhizodeposition affected the formation of the active microbial biomass in the soil-root interface, but there exists a range of complex interactions between the soil, microbiological and specific plant characteristics that needs further investigation.

Short-term pretreated municipal wastewater application to the abandoned agricultural soils had a weak positive effect on microbial communities. However, these soils were characterized by significantly higher structural and functional diversity and activity values compared to the other studied arable soil groups without wastewater treatment. Lower potential nitrification and high N-mineralization activity values for these groups may reflect the composition of the wastewater applied on the soil.

The microbial biomass was small, inefficient and its functional diversity was low in the domestic wastewater purifying sand filters of HSSFCW. The biggest microbial metabolic quotient among the studied soil groups suggested the dependence of these constructed systems on allochthonous nutrients and environmental factors. The microbial biomass was horizontally stratified in SF bodies. The activity and functional structure of the microbial communities of SFs was dependent on hydraulic conditions and concurrent oxygen and nutrient availability in filter bodies. Wet conditions supported higher activity and functional diversity values, and smaller differences between layers. Dry conditions resulted in greater differences in microbial functional structure between filter layers. The upper layer of dry SF was characterized by higher N- immobilization while in the lower layer this process was limited by the availability of oxygen. In wet conditions this process was possibly coupled with anaerobic processes (methanogenesis, etc.). The nitrification potential of the microbial communities in sand filters was bigger than in most groups of arable soils. In addition to the autotrophic nitrification also heterotrophic nitrification can contribute to this potential in these kinds of constructed systems.

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SUMMARY IN ESTONIAN

Maakasutuse mõju mikroobikooslustele Eesti muldades

Muld on keeruline ja kompleksne keskkond, milles mikroorganismid on olulised ainete ringluses osalejad, muutes orgaanilistesse ühenditesse seotud mineraalained teistele organismidele kättesaadavaks. Mulla mikroobid osalevad nii orgaanilise aine moodustamises (huumusaine, glomaliin) kui ka suuremate mullas sisalduvate orgaanilise aine molekulide lagundamises väiksemateks subühikuteks ning on võimelised lagundama ka erinevaid mürkaineid. See omadus võimaldab neid organisme edukalt ka erinevates keskkonnatehnoloogilistes protsessides rakendada. Teisalt on mikroorganismid tundlikud mitmesugustele keskkonnas toimuvatele muutustele, mille tõttu peetakse neid organisme headeks indikaatoriteks ka muldade seires. Majanduse kiire areng, seal hulgas ka üha intensiivistuv põllumajanduslik tootmine, ning sellest tulenev tarbimise kasv ja jäätmete hulga suurenemine, on põhjustanud suureneva surve mullale ning toonud esile muldade kvaliteedi säilitamise vajaduse. Vaatamata sellele, et põllumajandusliku tegevuse mõju muldade mikroobikooslustele on mujal maailmas (Lõuna- ja Kesk-Euroopa, USA) suhteliselt palju uuritud, on Balti regioon selles valdkonnas jäänud piisava tähelepanuta. Samuti on teadmised erinevate keskkonnatehnoloogiliste protsesside, nagu näiteks heitvee puhastus taimefiltrites ja kunstlikes märgalades, mõjust mulla mikroorganismidele endiselt äärmiselt lünklikud.

Käesoleva töö eesmärgiks oli hinnata erineva maakasutuse mõju haritavatele pedogeensete muldade ja antropomorfsete materjalide (nagu mahajäetud põlevkivikarjääride pinnas ning kunstliku horisontaalvoolulise märgala liivafiltrid) mikroobikooslusetele ning võrrelda vastavate muldade mikrobioloogilisi parameetreid loodulike pedogeensete muldade vastavate parameetritega. Mahajäetud põlevkivikarjääride pinnaste puhul analüüsiti eraldi erinevate puuliikidega (*Alnus incana*, *A. glutinosa*, *Picea abies*, *Betula pendula*, *Pinus sylvestris*) taimestatud ja taimestamata pinnaseid. Haritavate muldade hulgas vaadeldi aktiivseks põllumajanduslikuks tootmiseks kasutatavaid muldi, põllumajanduslikust kasutusest välja jäänud muldi, erinevate puuliikidega (*Salix* sp., *Alnus incana*, *A. glutinosa*, *Picea abies*, *Betula pendula*, *Pinus sylvestris*) taimestatud endiseid põllumuldi ja olmereovee järelpuhastuseks kasutatavaid nii taimestatud (*Salix* sp.) kui taimestamata endiseid põllumuldi. Maakasutuse mõju hindamiseks mulla mikroobikooslustele kasutati mikroobide biomassi (SIR ja PLFA analüüs) ning selle liigilist (DGGE ja PLFA analüüs) ja funktsionaalset mitmekesisust (BiologEco mikroplaatide alusel) ning erinevate ensüümide (aluseline ja happeline fosfataas, dehüdrogenaas) ja protsesside (mikroobne hingamine, nitrifikatsioon, lämmastiku mineralisatsioon) potentsiaalset aktiivsust. Mõõdetud parameetritest arvutati mikroobikoosluse mitmekesisust iseloomustavad (Shannon) indeksid ning koosluse stabiilsust iseloomustav metaboolne koefitsient (qCO_2). Maakasutuse mõju hindamiseks koostati erinevate uuringute

keemiliste, biokeemiliste ning mikrobioloogiliste analüüside tulemustest andmebaas (n = 178), mida kasutati andmeanalüüsis.

Tulemuste analüüs näitas, et maakasutus mõjutas oluliselt mulla mikrobioloogilisi ja biokeemilisi omadusi. Mikroobikoosluse vastus maakasutuse muutusele sõltus oluliselt mullatüübist ja kasvukohale omastest tingimustest. Haritavate muldade mikroobide biomass oli tunduvalt väiksem ja aktiivsus madalam kui looduslikes muldades. Kõige väiksem mikroobide biomass oli kunstliku märgala liivafiltrites ja mahajäetud karjääride pinnastes. Rekultiveeritud põlevkivikarjääride pinnaste mikroobikoosluste vastavad väärtused jäid looduslike ja haritavate muldade vahele.

Haritavatele muldadele olid iseloomulikud hästi kohastunud ja stabiilselt funktsioneerivad mikroobikooslused. Põllumajanduslik praktika koosneb reast üksikutest tegevustest (orgaaniliste ja/või anorgaaniliste väetistega väetamine, kündmine, viljavaheldus, pestitsiididega töötlemine jne.), mille mõju mulla mikroobikoosluse biomassile ja aktiivsusele oli spetsiifiline. Mineraalse lämmastikväetise pikaajaline kasutamine põhjustas mikroobide biomassi vähenemist mullas. Orgaaniliste väetiste kasutamine ja liblikõieliste taimede kasvatamine külvikorras suurendasid mikroobide biomassi ning tõstsid selle aktiivsust.

Muudatused maakasutuses peegeldusid ka mikroobikoosluse struktuuris ja aktiivsuses.

Põllumajanduslikust kasutusest välja jäänud muldadele ja mahajäetud karjääride pinnastele oli iseloomulik väike mikroobne biomass ja madal aktiivsus. Nende pinnaste taimestamine erinevate puuliikidega põhjustas biomassi tunduva suurenemise ning mikroobsete protsesside aktiviseerumise tänu taimejuurte toetusele (risodepositsioonile). Taimejuurte vahetus läheduses, risosfääris, oli mikroobikoosluse aktiivsus kõrgem ja funktsionaalne mitmekesisus suurem kui juurevabas mullas. Vähemsoodsates tingimustes, nagu mahajäetud karjääride ja endiste põllumuldade noortes lepapuistutes, olid erinevused risosfääri ja juurevaba mulla mikroobikoosluste aktiivsuste vahel suuremad võrreldes metsamuldade ning samade pinnaste vanemate lepapuistustega. Mikroobikoosluse kujunemist erinevate lepikute muldades mõjutasid oluliselt kasvukohaspetsiifilised tingimused. Pajude istutamine mahajäetud põllumuldadele suurendas mikroobikoosluse mitmekesisust mullas. Samal ajal võis täheldada erinevatelt katselappidelt võetud muldade mikroobikoosluste sarnasuse suurenemist peale pajuistanduse kolmanda kasvuperioodi lõppu võrreldes esimese kasvuperioodi lõpuga. Erinevate taimestatud pinnaste analüüsi tulemus näitas, et eriti kiire on juurevaba mulla mikroobide biomassi suurenemine puistu esimestel (1–9) kasvuaastatel.

Eelpuhastatud olmeheitveega kastmine mõjutas positiivselt haritavate muldade mikroobseid protsesse — suurendas mikroobikoosluse funktsionaalset ja liigilist mitmekesisust endistes põllumuldades. Olmeheitvee mõjul suurenes mulla kaaliumi kontsentratsioon ja tõusis mikroobikoosluse fosfataasne aktiivsus. Suurimad muutused toimusid ülemises, 0–10 cm, mullakihis.

Kunstliku märgala horisontaalsete liivafiltrite mikroobide biomassid olid oluliselt väiksemad pedogeensete muldade vastavate näitajatega võrreldes. Mikroobide biomassi horisontaalne jaotus filtris sõltus filtri hüdroloogilistest tingimustest. Suurim metaboolse koefitsiendi (mikroobse hingamise ja mikroobide biomassi suhte) väärtus uuritud gruppide hulgas osutas mikroobikoosluste jaoks ebastabiilsetele tingimustele kunstliku märgala liivafiltrites. Tingimustes, kus vajalike toitainete kättesaadavus sõltus kõikuvast heitvee tasemest filtris ning vajadusest kohaneda pidevalt muutuvate keskkonnatingimustega, olid mikroorganismid sunnitud kulutama tunduvalt rohkem energiat oma biomassi säilitamiseks. Mikroobikoosluse nitrifikatsiooni potentsiaal oli liivafiltrites oluliselt suurem kui enamuses haritavates muldades (välja arvatud põllumullad).

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APPENDIX

Tabel 1. Soil sampling data and measured parameters. Abbreviation: M — meadows, WM — wooded meadows, NW — natural wetlands, MM — anthropomorphic materials from mining area , SF — sand filters, WWT — wastewater treatment wetlands, SIR — active microbial biomass, PLFA — total microbial biomass, bPLFA — bacterial biomass, fPLFA — fungal biomass, BD — bacterial species diversity by DGGE, mSh — microbial community diversity by PLFA, bSh — bacterial diversity by PLFA, BA — Biolog activity, BS — functional diversity (Biolog Shannon indexes), resp — basal respiration, qCO_2 — metabolic quotient, DH — dehydrogenase activity, pot nit — potential nitrification activity, N-min — nitrogen mineralization activity, AlP — alkaline phosphatase activity, AcP — acid phosphatase activity, PE — pot experiment

Soil /material		Land use	Year of sampling	No. of fields/ beds	No. of samples	Measured / calculated parameters	References
Pedognic soils	Arable soils	Agriculture	2001	19	19	SIR, PLFA, bPLFA, fPLFA, BD, mSh, bSh, BS, BA, resp, qCO_2, DH, pot nit, N-min, AlP, AcP.	Unpublished data
			2003	23	27	SIR, mic C/N, BS, resp., qCO_2, DH, pot nit, N-min.	Truu et al. 2008a
		Abandoned	2003, 2005	1	13	SIR, BS, BA, qCO_2, resp, pot nit, N-min., AlP, AcP.	Truu et al. 2008b
			2003	2(PE)	2	SIR, BA, BS, resp, qCO_2, pot nit, N-min, AlP, AcP.	unpublished data
		Reclaimed	1999, 2004	2	2	SIR, BS, BA., resp, qCO_2	Lõhmus et al.2006
			2002	1	1	SIR, BS, BA., resp, qCO_2	Vares et al. 2004
			2003	2(PE)	6	SIR, BS, BA, resp, qCO_2, pot nit, N-min, AlP, AcP.	Unpublished data
			2003, 2005	1	16	SIR, BS, BA, resp, qCO_2, pot nit, N-min, AlP, AcP.	Truu et al. 2008b
			2006,	7	7	SIR, BD, BA, BS, resp, qCO_2	Unpublished data
		WW T	2005	1	3	SIR, BS, BA, qCO_2, resp, pot nit, N-min, AlP, AcP.	Truu et al. 2008b
		Reclaimed WWT		1	14		
	Natural soils	M	2006	4	4	SIR, resp, qCO_2, BD	Unpublished data
		WM		8	8		
		NW		17	17		
Anthtropomor-phic materials	MM	Abandoned	2003	1(PE)	1	SIR, BS, BA, resp, qCO_2, pot nit, N-min, AlP, AcP.	Unpublished data
			2002	3	3	SIR, BA, BS, resp, qCO_2	Vares et al. 2004
		Reclaimed	2003	1(PE)	3	SIR, BA, BS, resp, qCO_2, pot nit, N-min, AlP, AcP.	Unpublished data
			2000, 2002, 2004, 2006	11	12	SIR, BA, BS, resp, qCO_2	Lõhmus et al. 2005 Unpublished data
	SF	WW T	2001	2	24	SIR, BA, BS, resp, pot nit	Nurk et al. 2005

Table 2. Ratios of measured chemical parameters in studied soils with different land use. Abbreviations: AM — anthropomorphic materials, MM — mining AMs, WM — wooded meadow, NW — natural wetlands, SF — sand filter, Agricult. — agriculturally managed, Aband. — abandoned, Recl. — reclaimed, WWT — wastewater treated, SOM — soil organic matter, nd — not detected.

Para-meter	Pedogenic soils								AM		
	Arable soils					Natural soils			MM		SF
	Aband.	Agricult.	Recl.	WWT	Recl,WWT	Meadows	WM	NW	Aband.	Recl.	WWT
SOM (%)	2.13–5.92	2.04–11.2	1.94–26.7	2.82–3.01	2.76–6.21	8.74–13.61	7.69–14.7	4.76–27.4	1.41–7.19	1.30–15.0	0.22–0.69
N (%)	0.10–0.29	0.10–0.57	0.74–1.19	0.12–0.13	0.12–0.22	0.49–0.77	0.34–0.70	0.22–1.43	0.03–0.03	0.02–0.55	0.01–0.90
P (mg/kg)	6.45–84.2	17.3–683	6.57–75.4	74.2–81.2	41.2–75.2	45.7–260.6	10.3–61.9	16.5–112	14.9–73.5	12.4–130.8	30.2–192
K (mg/kg)	33.4–171	55.0–397	19.6–133	214–247	34.1–184	184 –264	79.3–251	18.3–356	191–227	nd	nd
pH	4.00–7.23	4.62–7.52	3.76–7.25	4.58–4.95	4.36–5.26	6.86–7.06	5.54–7.01	5.01–7.26	7.90–8.12	6.83–7.92	6.7–7.2

Table 3. Values of measured microbiological parameters (means, standard deviations and numbers of samples analysed) for studied soil groups and anthropomorphic materials (AM) with different land use. Abbreviations: MM — mining AMs, SF — sand filter, Aband. — abandoned, Agricult. — agriculturally managed, Recl. — reclaimed, WWT — wastewater treated, M — meadows, WM — wooded meadows,
NW — natural wetland, SIR — active microbial biomass, PLFA — total microbial biomass, bPLFA — bacterial biomass, fPLFA — fungal biomass, Biom-N — microbial biomass-N, Biom C/N — microbial biomass-C and N ratio, qCO_2 — metabolic quotient, pot nit — potential nitrification activity, N-min — N mineralization activity, DH — dehydrogenase activity, AcP — acid phosphatase activity, AlP — alkaline phosphatase activity, BA — Biolog activity, BS — functional diversity (Shannon index) by Biolog, mSh — microbial community diversity by PLFA, BD — bacterial diversity by DGGE, bSh — bacterial diversity by PLFA, TPF — triphenyl formazan, NP- p-nitrophenol, OD — optical density, dw — dry weight, nd — not detected.

Parameter	Pedogenic soils								AM		
	Arable soils					Natural soils			MM		SF
	Aband.	Agricult.	Recl.	WWT	Recult.,WWT	M	WM	NW	Aband.	Recl.	WWT
SIR mg biomC g^{-1} dw	0.30±0.17 (n=15)	0.53±0.20 (n =44)	0.54±0.38 (n=30)	0.61±0.02 (n=3)	0.65±0.14 (n=14)	3.24±0.92 (n=4)	2.11±0.74 (n=8)	1.52±0.71 (n=17)	0.02±0.10 (n=4)	0.91±0.93 (n=15)	0.11±0.01 (n=24)
PFLA µg C^{g-1}dw	nd	0.97±0.49 (n=17)	nd	nd	nd	nd	nd	nd	nd	nd	nd
bPLFA µg C g^{-1} dw	nd	0.73±0.32 (n=17)	nd	nd	nd	nd	nd	nd	nd	nd	nd
f PLFA µg C g^{-1} dw	nd	0.02±0.01 (n=17)	nd	nd	nd	nd	nd	nd	nd	nd	nd
Biom-N mgCg^{-1}dw	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.02±0.01 (n=24)
Biom C/N	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	7.44±5.77 (n=24)
Respiration mg CO_2 g^{-1} dw $24h^{-1}$	0.07±0.05 (n=15)	0.11±0.06 (n=44)	0.12±0.08 (n=30)	0.12±0.01 (n=3)	0.13±0.03 (n=14)	0.63±0.11 (n=4)	0.47±0.20 (n=8)	0.46±0.19 (n=17)	0.08±0.04 (n=4)	0.33±0.31 (n=15)	0.05±0.01 (n=24)
qCO_2	0.29±0.09 (n=15)	0.21±0.10 (n=44)	0.26±0.07 (n=30)	0.19±0.02 (n=3)	0.21±0.05 (n=14)	0.20±0.03 (n=4)	0.23±0.08 (n=8)	0.32±0.12 (n=17)	0.62±0.40 (n=4)	0.44±0.15 (n=15)	0.92±0.26 (n=24)

Parameter	Pedogenic soils								AM		
	Arable soils					Natural soils			MM		SF
	Aband.	Agricult.	Recl.	WWT	Recult.,WWT	M	WM	NW	Aband.	Recl.	WWT
Pot nit µg Ng^{-1} dw $24h^{-1}$	0.40±0.84 (n=15)	2.95±3.45 (n=44)	0.59±1.16 (n=21)	0.16±0.15 (n=3)	0.17±0.12 (n=14)	nd	nd	nd	0.05 (n=1)	0.03±0.01 (n=3)	0.60±0.48 (n=24)
N-min µg Ng^{-1} dw $24h^{-1}$	1.32±0.56 (n=15)	1.06±0.58 (n=44)	1.20±0.50 (n=21)	2.50±0.15 (n=3)	2.01±0.37 (n=14)	nd	nd	nd	0.048 (n=1)	0.06±0.03 (n=3)	nd
DH µgTPFg^{-1} dw h^{-1}	nd	4.67±3.23 (n=44)	nd	nd	nd	nd	nd	nd	nd	nd	nd
AcP µg NPg^{-1} dw h^{-1}	277±49.7 (n=15)	239±84.0 (n=44)	261±53.2 (n=21)	295±14.7 (n=3)	330±113 (n=14)	nd	nd	nd	21.0 (n=1)	21.9±7.61 (n=3)	nd
AlP µg NPg^{-1} dw h^{-1}	77.8±168 (n=15)	358±266 (n=44)	116±228 (n=21)	26.8±11.8 (n=3)	58.6±20.1 (n=14)	nd	nd	nd	102.1 (n=1)	80.6±12.8 (n=3)	nd
BA ODg^{-1} dw	8.58±2.02 (n=15)	10.1±3.16 (n=44)	7.99±3.97 (n=30)	10.38±1.07 (n=3)	9.05±2.56 (n=14)	nd	nd	nd	2.65±0.19 (n=3)	6.54±5.79 (n=15)	nd
BS	2.80±0.10 (n=15)	2.71±0.19 (n=44)	2.80±0.16 (n=30)	2.93±0.02 (n=3)	2.88±0.09 (n=14)	nd	nd	nd	2.31±0.24 (n=4)	2.76±0.22 (n=15)	2.60±0.43 (n=24)
mSh (PLFA)	nd	2.52±0.05 (n=17)	nd	nd	nd	nd	nd	nd	nd	nd	nd
BD (DGGE)	3.37±0.18 (n=12)	3.19±0.11 (n=10)	3.23±0.33 (n=22)	3.41±0.05 (n=3)	3.43±0.07 (n=12)	3.24±0.13 (n=4)	3.04±0.40 (n=8)	3.22±0.35 (n=17)	nd	nd	nd
bSh (bPLFA)	nd	2.42±0.03 (n=17)	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 4. Statistically significant Spearman rank order correlations between microbiological and chemical parameters for all studied soils. Abbreviations: SOM — soil organic matter, SIR — active microbial biomass, Resp — basal respiration, Pot nit — potential nitrification activity, AlP — alkaline phosphatase activity.

Chemical parameter	SIR	Resp	Pot nit	AlP
N%	0.81*** n=181	0.71*** n= 181	–	0.63*** n= 104
SOM(%)	0.80*** n=181	0.80*** n= 181	–	0.61*** n= 104
pH_{KCl}	–	–	0.67*** n=104	–
P mg kg^{-1}	–	–	0.52*** n= 128	–
K mg kg^{-1}	–	–	0.49*** n= 92	–

*** P<0.0001

Table 5. Statistically significant Spearman rank order correlations between microbiological and chemical parameters for arable soils under agriculture. Abbreviations: SOM — soil organic matter, SIR — substrate induced respiration, PLFA — total microbial biomass, bPLFA — bacterial biomass, fPLFA — fungal biomass, Resp — basal respiration, Pot nit — potential nitrification activity, N-min — nitrogen mineralization activity, DH — dehydrogenase activity, AlP — alkaline phosphatase activity, BA — Biolog activity, bSh — bacterial diversity by PLFA.

Chemical parameter	SIR	PLFA	bPLFA	fPLFA	Resp	Pot nit	N-min	DH	AlP	BA	bSh
N %	0.62 ***	0.94 ***	0.95 ***	0.83 ***	0.65 ***	0.79 ***	0.45 *	0.90 ***	0.89 ***	0.49 *	–0.60 ***
SOM %	0.57 ***	0.89 ***	0.89 ***	0.74 ***	0.59 ***	0.69 ***	–	0.84 ***	0.84 ***	0.47 *	–0.49 *
pH_{KCl}	0.65 ***	0.67 *	0.70 **	0.80 ***	0.76 ***	0.58 ***	–	0.72 *	0.78 ***	–	–0.66 *

* P<0.05, ** P<0.001, *** P<0.0001

Table 6. Statistically significant Spearman rank order correlations between microbiological and chemical parameters for abandoned arable soils. Abbreviations: SOM — soil organic matter, Resp — basal respiration, Pot nit — potential nitrification activity, N-min — nitrogen mineralization activity, AcP — acid phosphatase activity, BS — functional diversity.

Chemical parameter	Resp	Pot nit	N-min	AcP.	BS
N %	–	–	0.64 *	0.65 *	–
SOM %	–	–	0.52 *	0.69 *	–
P mg kg^{-1}	–	–	–	–	0.64 *
K mg kg^{-1}	0.64 *	0.60 *	0.52 *	–	–

* P<0.05, ** P<0.001, *** P<0.0001

Table 7. Statistically significant Spearman rank order correlations between microbiological and chemical parameters for reclaimed arable soils. Abbreviations: SOM — soil organic matter, qCO_2 — metabolic quotient, Pot nit — potential nitrification activity, N-min — nitrogen mineralization activity, AcP — acid phosphatase activity, BS — functional diversity, BD — bacterial diversity by DGGE.

Chemical parameter	qCO_2	Pot nit	N-min	AcP.	BS	BD
N%	–	–	–	0.47 *	–	–
SOM %	–	–	0.50 *	–	–	–
pH_{KCl}	–	0.70 **	–	–	–	–
P mg kg^{-1}	–	–	0.59 *	0.59 *	–	–0.54 *
K mg kg^{-1}	0.52 *	0.54 *	0.70 *	–	0.55 *	–

* P<0.05, ** P<0.001

Table 8. Statistically significant Spearman rank order correlations between chemical and microbiological parameters in reclaimed and wastewater treated arable soils. Abbreviations: SOM — soil organic matter, SIR — active microbial biomass, Resp — basal respiration, Pot nitr — potential nitrification activity, AcP — acid phosphatase activity, AlP — alkaline phosphatase activity, BS- functional diversity.

Chemical parameter	SIR	Resp	Pot nitr	AcP	AlP	BS
N %	0.53 *	0.55 *	–	0.97 **	0.82 **	0.63 *
SOM %		0.56 *	–	0.83 **	0.67 *	0.61 *
pH_{KCl}	–	–	0.61 *	–	–	–

* P<0.05, ** P<0.001

Table 9. Statistically significant Spearman rank order correlations between chemical and microbiological parameters for reclaimed mining anthropomorphic materials. Abbreviations: SOM — soil organic matter, SIR — active microbial biomass, Resp — basal respiration, BS — functional diversity.

Chemical parameter	SIR	Resp	BS
N %	0.69 *	0.91 ***	0.86 ***
SOM %	0.60 *	0.64 *	0.65 *
pH_{KCl}	–0.67 *	–0.86 ***	–0.84 ***

* P<0.05, *** P<0.0001

Table 10. Statistically significant Spearman rank order correlations between chemical and microbiological parameters for sand filters of HSSFCW. Abbreviations: SOM — soil organic matter, Biom-N — microbial N (immobilized N), BiomC/N — microbial biomass-C and N ratio, BS — functional diversity.

Chemical parameter	Biom-N	Biom C/N	Pot nitr	BS
N %	0.75 ***	–0.71 **	–0.54 *	–
SOM %	0.71 **	–0.57 *	0.74 ***	0.42 *
P $mg\ kg^{-1}$	–	–	0.60 *	–

* P<0.05, ** P<0.001, *** P<0.0001

Table 11. Statistically significant Spearman rank order correlations between chemical and microbiological parameters for natural wetlands soils. Abbreviations: SIR — active microbial biomass, Resp — basal respiration, qCO_2 — metabolic quotient.

Chemical parameter	SIR	Resp	qCO_2
pH	–	0.58 *	0.52 *
K mg kg^{-1}	–0.54 *	–	–

* P<0.05

Table 12. Statistically significant Spearman rank order correlations between chemical and microbilogical parameters for wooded meadow soils. Abbreviations: SOM — soil organic matter, SIR — active microbial biomass, Resp — basal respiration.

Chemical parameter	SIR	Resp
N%	–	0.79 *
SOM %	–	0.74 *
P mg kg^{-1}	0.71 *	0.95 **

* P<0.05, ** P<0.001

Table 13. Statistically significant Spearman rank order correlations of total (PFLA, n=20) and active (SIR, n=48) microbial biomass, fungal (fPFLA, n=20) and bacterial (bPFLA, n=20) biomass with measured community structure and activity parameters in agriculturally managed arable soils. Abbreviations: Resp — basal respiration activity, N-min — N-mineralization, Pot nit — potential nitrification, DH — hedydrogenase activity, AcP — acid phosphatase activity, AlP — alkaline phosphatase activity, qCO_2 — metabolic quotient, BA- activity by Biolog , BS — functional diversity (Shannon) by Biolog, bSh — bacterial diversity (Shannon indexes by bPFLA).

Biomass	Resp	N-min	Pot nit	DH	AcP	AlP	qCO_2	BA	BS	bSh
SIR	0.67 ***	0.62 ***	0.58 ***	0.74 ***	–	0.65 ***	–	–	–	–0.58 *
PFLA	0.74 **	0.69 **	0.80 ***	0.88 ***	0.61 *	0.90 ***	–	0.62 *	0.65 *	–0.51 *
bPFLA	0.74 **	0.69 **	0.81 **	0.88 ***	0.59 *	0.90 ***	–	0.62 *	0.66 *	–0.55 *
fPFLA	0.84 ***	0.68 *	0.84 ***	0.83 ***	–	0.92 ***	0.52 *	0.68 **	0.68 **	–0.67 *

*P<0.05, **P<0.001, ***P<0.0001

13

PUBLICATIONS

CURRICULUM VITAE

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1996–1999	MSc (Environmental protection) from Estonian University of Life Science

Additional studies

1997	Agricultural landscapes (Häme Polytecnic, Finland)
1999	Biochemical analyses in soil microbial ecology (Laboratory of Soil Biology, Institute of Soil Science, Hohenheim University, Germany)
2002	Management and Treatment of Polluted Soils in Europe (Socrates Intensive Programme, Tartu, Estonia)
2003	Molecular analyses in soil microbial ecology (Laboratory of Soil Biology, Institute of Soil Science, Hohenheim University, Germany)

Professional employment

1996–1999	Environmental Protection Institute, Estonian University of Life Science, Microbiologist
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DISSERTATIONES TECHNOLOGIAE CIRCUMIECTORUM UNIVERSITATIS TARTUENSIS

1. **Sille Teiter.** Emission rates of N_2O, N_2, CH_4 and CO_2 in riparian grey alder forests and subsurface flow constructed wetlands. Tartu, 2005.
2. **Kaspar Nurk.** Relationships between microbial characteristics and environmental conditions in a horizontal subsurface flow constructed wetland for wastewater treatment. Tartu, 2005.
3. **Märt Öövel.** Performance of wastewater treatment wetlands in Estonia. Tartu, 2005.
4. **Alar Noorvee.** The applicability of hybrid subsurface flow constructed wetland systems with re-circulation for wastewater treatment in cold climates. Tartu, 2005.
5. **Christina Vohla.** Phosphorus removal by various filter materials in subsurface flow constructed wetlands. Tartu, 2008.
6. **Martin Maddison.** Dynamics of phytomass production and nutrient standing stock of cattail and its use for environment-friendly construction. Tartu, 2008.