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TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

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

I   **Espenberg M**, Truu M, Truu J, Maddison M, Nõlvak H, Järveoja J, Mander Ü (2016) Impact of reed canary grass cultivation and mineral fertilisation on the microbial abundance and genetic potential for methane production in residual peat of an abandoned peat extraction area. *PLoS ONE* 11(9): e0163864.

II   Abdalla M, Hastings A, Truu J, **Espenberg M**, Mander Ü, Smith P (2016) Emissions of methane from northern peatlands: a review of management impacts and implications for future management options. *Ecology and Evolution* 6: 7080–7102.

III   **Espenberg M**, Truu M, Mander Ü, Kasak K, Nõlvak H, Ligi T, Oopkaup K, Maddison M, Truu J (201X) Response of microbial community structure and nitrogen cycling to drainage in a tropical peatland soil. (submitted)

IV   Truu J, Truu M, **Espenberg M**, Nõlvak H, Juhanson J (2015) Phyto-remediation and plant-assisted bioremediation in soil and treatment wetlands: a review. *The Open Biotechnology Journal* 9: 85–92.

Author's contribution

Publication I: The author performed sampling (50%), all the microbiological analyses (100%) and most of the data analyses (95%) and is responsible for writing the manuscript (about 75%).

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Publication III: The author performed microbiological analyses (40%), most of the data analyses (95%) and is responsible for writing the manuscript (about 80%).

Publication IV: The author is responsible for writing the manuscript (about 10%).

ABSTRACT

Peatlands, which are widespread ecosystems in the world, have a critical role in regulating the carbon and nitrogen cycles at regional and global scales. During recent centuries, these ecosystems have faced increasing pressures that have arisen from anthropogenic events. In this dissertation, the effects of a range of peatland management practices are evaluated in order to study alterations in soil microbiome and greenhouse gas emissions.

A total of 87 studies conducted at 186 sites covering different countries, peatland types and management systems were studied to investigate the impact of different factors (including management practices) on methane (CH_4) emissions in northern peatlands (latitude 40° to 70°N). Results show that the overall annual average of CH_4 emissions from natural northern peatlands is 12 ± 21 g C m^{-2} year^{-1} and it is highly variable with a 95% confidence interval of 7.6–15.7 g C m^{-2} year^{-1} for the mean. Compared to bogs, fens emit higher levels of CH_4 to the atmosphere. CH_4 emissions from natural peatlands are mainly controlled by water table depth, plant community composition and soil pH. Maximum emissions occur when mean annual air temperature is approximately 2°C. Although the mean annual air temperature is not a good predictor of CH_4 emissions by itself, the interactions between temperature, plant community cover, water table depth and soil pH are important. According to the short-term forecasts of climate change, these complex interactions will be the main determinants of CH_4 emissions from the northern peatlands. The drainage of northern peatlands clearly reduces the CH_4 flux, on average, by 84% compared to the original emission values with a mean of 8.3 g C m^{-2} year^{-1}.

Greenhouse gas emissions were also affected by drainage in a tropical peatland. The average CH_4 flux from the natural site was higher than that for the drained (35.8 ± 24.5 g C m^{-2} year^{-1} and 0.05 ± 0.07 g C m^{-2} year^{-1}, respectively) and the average nitrous oxide (N_2O) flux from the natural site was lower than that for the drained (0.003 ± 0.015 and 0.09 ± 0.10 g N m^{-2} year^{-1}, respectively). Additionally, the potential for dinitrogen (N_2) emission was greater in the top 10-cm soil layer at the natural site compared to the drained site (12.9 ± 5.5 and 7.1 ± 3.8 g N m^{-2} year^{-1}, respectively). Drainage changed the balance between bacterial and archaeal groups in the peat in favour of plant biomass-decomposing microbial communities, especially towards the phyla *Actinobacteria*, *Firmicutes* and *Crenarchaeota*, and euryarchaeal class *Thermoplasmata*. In addition, the drainage of the tropical peatland led to other structural changes in the soil bacterial and archaeal community and resulted in alterations in *nirK*, *nirS*, *nosZ*, *nifH* and archaeal *amoA* gene-possessing microbial communities. The denitrification and N_2-fixing potential in the tropical peatland were reduced by the drainage. In natural peatland soil, the N_2O emission was primarily related to *nirS*-type denitrifiers and dissimilatory nitrate reduction to ammonium, while the conversion of N_2O to N_2 was controlled by *nosZ* clade I genes. The drainage induced changes in the soil denitrifying

microbial community and the main reducers of N_2O were microbes harbouring *nosZ* clade II genes.

Restoration of drained northern peatlands by rewetting or simultaneous vegetation and rewetting increases CH_4 emissions by an average of 1.3 ± 6.5 g C m^{-2} year^{-1} (46%), although the change in CH_4 flux due to rewetting was not statistically significant (mean flux 3.0 ± 3.1 g C m^{-2} year^{-1} before restoration and 4.2 ± 6.3 g C m^{-2} year^{-1} after restoration). Different management practices and initial peatland type determines a response to rewetting. Energy crop (reed canary grass) cultivation in residual peat of an abandoned peat extraction area reduced CH_4 emissions slightly, although methanogen abundance remained approximately the same or even increased in different layers of residual peat under cultivated sites over time. In general, the methanogen abundance was low in the archaeal community, but the highest CH_4 production potential was revealed at the bottom of the peat column of the abandoned peat extraction area. In uncultivated peat, the two deeper layers influenced mainly the CH_4 emission, while in cultivated peat, the more pronounced effect of methanotrophic bacteria on CH_4 emission can be assumed from the obtained results. Additionally, the physicochemical status of peat had slightly changed and bacterial abundance increased in response to the reed canary grass cultivation.

Peatlands have a good potential to improve water treatment at a fairly low cost in peatland-rich regions in order to protect aquatic ecosystems from pollution. Natural treatment wetlands have been used to treat many different contaminants including excessive nitrogen, phosphorus, suspended solids, sulphur and metals. Besides peat, vegetation type and density also affect the compounds retention efficiency from treated water.

This dissertation concludes from the results that management practises affect form and function of peatlands by changing considerably the soil microbiome and influencing greenhouse gas emissions from peat, although some management practices alleviate environmental damage.

1. INTRODUCTION

1.1 Soil biogeochemical processes and greenhouse gas emissions in peatlands

Peatlands are the most widespread type of wetlands (50–70%) in the world that cover about 3% of the world's land area. These unique ecosystems, where continuous vegetation production and slow decomposition rates under water-logged conditions lead to a very high content of organic matter in soils, store ⅓ of the world's soil carbon. The majority of the world's peatlands are located in the boreal and temperate regions, although these ecosystems occur from tropics to Arctic zones (Joosten & Clarke 2002). Approximately 11% of global peatlands are in tropical areas and they contribute up to 18–25% of the global peat carbon pool through both their aboveground biomass and underlying thick deposits of peat (Page et al. 2011). However, the results from recent studies suggest that far more peat exists in the tropics than was previously estimated (Dargie et al. 2017, Gumbricht et al. 2017), and both the tropical peat area and volume may be more than three times larger from previous estimates (Gumbricht et al. 2017). Furthermore, Voigt et al. (2017) showed that the thawing of permafrost in the Arctic peatlands is exposing a large stock of both carbon and nitrogen for active use in the biosphere.

In addition to the importance of the peatlands as carbon storage, they are also important ecosystems in terms of nitrogen cycling that have recently received great attention (Hatano et al. 2016, Koskinen et al. 2017, Voigt et al. 2017). Both cycles have great environmental, ecological and economical importance in the light of climate change and anthropogenic actions (e.g. land use change) (Galloway et al. 2008, Gruber & Galloway 2008). Soil carbon and nitrogen are the central components to maintain soil fertility and productivity and can substantially affect climate through carbon and nitrogen emissions (including carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O)) (Limpens et al. 2008, Sjögersten et al. 2011). Both CH_4 and N_2O have a strong negative effect on global warming. According to the IPCC report (2013), CH_4 has a global warming potential of 34 relative to CO_2 over a 100-year period (ca 20% of anticipated warming) and N_2O has a global warming potential of 298 relative to CO_2 over a 100-year period (ca 6% of anticipated warming).

In peatlands, the carbon and nitrogen cycles are closely related (Figure 1) and the quantity and distribution of both these nutrients are controlled through biogeochemical processes, where microbial communities play a key role (Lin et al. 2014, Mandic-Mulec et al. 2014). Any fluctuations in levels of these nutrients exert significant effects on the carbon and nitrogen cycles at regional and global scales (Mitsch & Gosselink 2015). Recognising the factors that affect different processes of both cycles is crucial to understanding the complex system in peatlands.
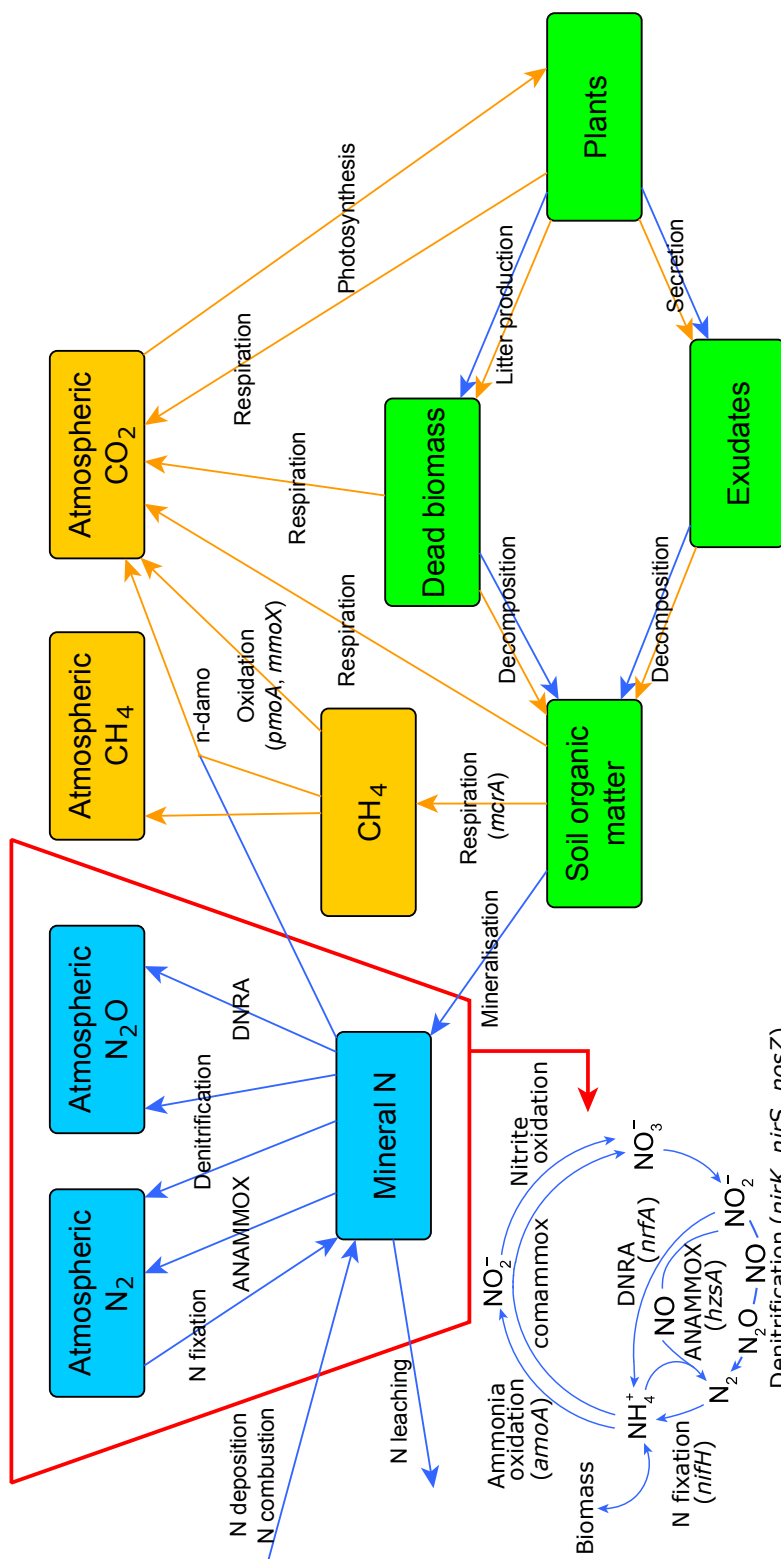
Figure 1. Schematic view of the coupled biogeochemical cycles of carbon (C) and nitrogen (N) in peatlands. Coloured arrows show the transfer of elements (C in orange and N in blue) between ecosystem compartments. Some important functional genes involved in these cycles are shown in brackets. Abbreviations: ANAMMOX – anaerobic ammonium oxidation, comammox – complete oxidation of ammonium to nitrate, DNRA – dissimilatory nitrate reduction to ammonium, n-damo – nitrite-dependent anaerobic methane oxidation.

A broad range of microorganisms found in peatlands have developed physiological and metabolic adaptations to survive constraining conditions (e.g. low oxygen availability and acidity) in these ecosystems (Andersen et al. 2013). Compared to northern peatlands, the soil microbial community structure in tropical peatlands is still poorly studied (Sjögersten et al. 2011). However, some studies have shown that northern and tropical acidic peatlands display similar patterns of bacterial diversity in the surface layers (0–40 cm depth), where the dominant phyla were *Acidobacteria* and *Proteobacteria*, and less abundant but numerically significant groups were *Verrucomicrobia*, *Actinobacteria* and *Planctomycetes* (Dedysh 2011). That kind of microbial community composition must have an advantage in these ecosystems. Several representatives of *Proteobacteria* play an important role in the carbon, nitrogen and sulphur cycles (Kersters et al. 2006). Furthermore, members of *Acidobacteria* have a broad range of substrate transporters for nutrient uptake suggesting advantage in complex environments and adaptation to oligotrophic conditions (Kielak et al. 2016), while *Actinobacteria* have shown to be greatly involved in the degradation and mineralisation of plant and humic materials in soil (Lewin et al. 2016). Additionally, many members of *Planctomycetes* contribute to the nitrogen cycle (Fuerst & Sagulenko 2011) and *Verrucomicrobia* play an important role in the carbon cycle (Herlemann et al. 2013), especially in soils where their proportion in the total bacterial community can be more than 20% (Bergmann et al. 2011).

Archaea from phyla *Euryarchaeota* and *Crenarchaeota* participate in carbon, nitrogen and sulphur cycles, *Korarchaeota* are more associated with the carbon cycle and *Thaumarchaeota* with nitrogen cycle (Offre et al. 2013). The activity of microbes involved in the carbon and nitrogen cycles depends on substrate specialisation and environmental variables (Andersen et al. 2013).

1.1.1 Carbon cycle

The carbon cycle is a circulation of carbon, a life-sustaining element, in various forms through sedimentary rocks, oceans, terrestrial ecosystems and atmosphere (Dignac et al. 2017). Presently, the balance of this element is changing between the ecosystems, and atmospheric concentration of carbon-containing greenhouse gases (CO_2, CH_4) is increasing, which is associated with climate change (IPCC 2013). Terrestrial ecosystems play a critical role in the soil-atmosphere carbon exchange system (Davidson & Janssens 2006). The flux of carbon in soils is initiated by the fixation of atmospheric CO_2 by photosynthesis. During this process, CO_2 is absorbed and converted into biomass of photosynthetic organisms, although some fraction of the previously captured CO_2 is released back to the atmosphere through respiration. Additionally, photosynthetic organisms release some carbon as organic compounds into the soil. Synthesised substances, microorganisms and residues of plant and animal are the organic matter components of soil (soil organic matter – SOM), which is the most

prevalent form of carbon in the soil (Dignac et al. 2017). Some organic compounds (e.g. root exudates) are readily mineralised after entering the soil, while others can persist in form of SOM for long periods (Schmidt et al. 2011). Physical mechanisms (e.g. sorption/desorption, diffusion, transport) can limit microbial access to substrates, therefore, interactions between microbes and substrates and soil physical conditions regulate carbon utilisation. Available substrates are not only converted to microbial biomass, but some carbon is returned to the atmosphere via respiration and it is also used for synthesis of a variety of organic products (e.g. extracellular enzymes and polysaccharides) that affect the functioning of ecosystems (Schimel & Schaeffer 2012).

If oxygen is scarce as generally it is in natural peatlands, some part of organic carbon is also released as CH_4 (Bridgham et al. 2013). Methanogenic archaea and methanotrophic bacteria, which determine soil exchange of CH_4 with the atmosphere, are mainly active in anaerobic and aerobic conditions, respectively (Aronson et al. 2013). All methanogens that have been charac-terised so far possess *mcrA* gene, which encodes the alpha-subunit of the methyl coenzyme M reductase – the enzyme that catalyses the last step in the CH_4 synthesis converting the fermentation end products (e.g. H_2/CO_2 and acetate) to CH_4 (Juottonen et al. 2006, Bridgham et al. 2013). The first step in the oxidation of CH_4 to CO_2 is the conversion of CH_4 to methanol by the particulate or soluble methane monooxygenase (pMMO/sMMO). Nearly all methanotrophs possess *pmoA* gene, encoding a subunit of pMMO, whereas *mmoX* gene (sMMO) is present only in a few methanotrophic genera (e.g. *Methylocella*) (McDonald et al. 2008). The CH_4-cycling microbial community structure varies between ecosystems and is affected by climate and environmental parameters as well as vegetation (Aronson et al. 2013).

1.1.2 Nitrogen cycle

Nitrogen is the most abundant element in the atmosphere and is also a vital element for all organisms as a component of proteins and nucleic acids. Nitrogen transformation processes are mainly mediated by microorganisms in soil and recent findings of new processes and organisms unfold new insights into the complex system of nitrogen cycling (Stein & Klotz 2016).

Autotrophic nitrification, the aerobic oxidation of ammonium or ammonia to nitrate via nitrite, was previously considered a two-step process catalysed by two different phylogenetically defined groups of microorganisms oxidising either ammonia or nitrite (Holmes et al. 1995, Könneke et al. 2005). Biological ammonia oxidation is controlled by the enzyme ammonia monooxygenase (AMO), where the alpha (A) subunit is encoded by the *amoA* gene, in both bacteria and archaea. Still, bacterial *amoA* genes are only distantly related to the archaeal *amoA* genes (Stahl & de la Torre 2012). The *amoA* gene has been used broadly as a marker for both ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA) in environmental studies (Oton et al. 2016). In many

habitats (including acidic soils and hot springs) AOA are shown to outnumber AOB (Hatzenpichler 2012, Oton et al. 2016, Pajares & Bohannan 2016), because of their highly efficient anabolic pathways that provide an ecological advantage relative to the AOB in multiple environments (Könneke et al. 2014). Thus, the discovery of AOA, which are categorised into a novel archaeal phylum *Thaumarchaeota*, fundamentally revised our understanding of nitrification (Hatzenpichler 2012). In 2015, the novel species of complete ammonia oxidizers were discovered in the nitrite-oxidising bacterial genus *Nitrospira*, who are capable of performing complete oxidation of ammonium to nitrate on its own (comammox) (Daims et al. 2015, van Kessel et al. 2015). They have been found from many different ecosystems (e.g. engineered treatment systems, rice paddy soil, forest soil), but their ecological background and relevance in the whole nitrogen cycle is still unclear (Daims et al. 2015, van Kessel et al. 2015, Pjevac et al. 2016). In addition to the autotrophic nitrification, heterotrophic nitrification can be a nitrogen oxidation process in soil (Zou et al. 2016), however, its relevance in the whole nitrogen cycling in different soils is not well known.

In addition to the heterotrophic nitrogen transformation processes that utilise organic matter as a carbon source, nitrite-dependent anaerobic methane oxidation (n-damo) is a process where nitrogen and carbon cycles are closely linked (Raghoebarsing et al. 2006). In this process, nitrite is reduced to nitric oxide that is further dismutated to dinitrogen (N_2) gas and oxygen at anoxic conditions. The produced oxygen is used as an electron acceptor to oxidise CH_4. Currently, there is only one bacterial species ("*Candidatus* Methylomirabilis oxyfera") conducting the described process (Ettwig et al. 2010). This organism is found in very different environments including wetlands (Ligi 2015) and peatlands (Zhu et al. 2012), but the information about ecological demand of this type of organisms is very limited.

Denitrification is a three or four step nitrogen reduction process, where nitrate is reduced to end products N_2O or N_2 gas (Wrage et al. 2001, Shoun et al. 2012). This process is carried out mainly by facultative anaerobic organisms that prefer oxygen as an electron acceptor when oxygen is present in the environment. Each of the reduction steps is catalysed by respective enzymes, i.e. periplasmic (Nap) or membrane-bound (Nar) nitrate reductases, nitrite reductases (CuNir and cd_1Nir), nitric oxide reductases (cNor, qNor and qCuANor) and nitrous oxide reductases (Nos) encoded by *nap* and *nar*, *nirK* and *nirS*, *nor* and *nosZ* genes, respectively (Zumft 1997). Microorganisms may carry out only part of the described pathway (Zumft 1997) and only one third of the organisms harbouring denitrification genes are shown to have a complete set of *nir*, *nor* and *nosZ* genes (Graf et al. 2014). Those denitrifying organisms, which lack the *nosZ* gene, are emitters of N_2O, whereas the others which have been shown to possess only the *nosZ* gene, can only reduce N_2O to N_2 (Jones et al. 2008, Sanford et al. 2012, Graf et al. 2014). A wide taxonomic range of different denitrifiers have a ubiquitous distribution in various environments, whereas denitrification is also a major cause of nitrogen loss in different soils

(Jones et al. 2008). Recent studies have shown that some denitrifiers retain their nitrogen reduction ability also in aerobic conditions in soil (Wang et al. 2017). This process is shown to be coupled with heterotrophic nitrification $(NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2)$ in several bacteria (Chen et al. 2012, Zhang et al. 2012, Zou et al. 2016, Zhang et al. 2017).

In contrast to denitrification, which is usually a major microbial pathway for nitrate reduction, dissimilatory nitrate reduction to ammonium (DNRA) may conserve nitrogen in the ecosystem as nitrate is transferred via nitrite to ammonium (Tiedje 1988). The key step in this process is related to the nitrite reductase enzyme NrfA encoded by *nrfA* gene (Welsh et al. 2014). As a result, nitrogen is more easily available for plant and microbial uptake as well as less prone to losses via leaching or as gaseous compounds (Tiedje 1988). But, depending on the environmental conditions, organisms capable of DNRA may release N_2O as a by-product of the reduction process or reduce N_2O that they produced themselves or provided by other microorganisms (Mania et al. 2014). DNRA is expected to be favoured in nitrate limited environments, with availability of a suitable organic carbon source, particularly in competition with denitrifiers. The capability for DNRA is widely spread among bacteria (Rütting et al. 2011). Contrary to decades of studies focused on denitrification, *nrfA*-containing organisms have received less attention and their contribution to nitrogen retention is vague (Welsh et al. 2014).

Besides DNRA, which is generally considered as a process that conserves nitrogen in the ecosystem (Rütting et al. 2011, Mania et al. 2014), biological nitrogen fixation is another process that promotes nitrogen retention in soils (van Groenigen et al. 2015). Virtually limitless supply of atmospheric N_2 is available to a rather small but diverse set of bacteria and archaea who can either symbiotically with host plant or free-livingly fix N_2 into biologically available ammonium (Reed et al. 2011). This reaction is catalysed by the reductase subunit of nitrogenase encoded by *nifH* gene (Zehr et al. 2003). The composition and abundance of N_2-fixers may be affected by a wide variety of abiotic and biotic factors in different ecosystems (agricultural lands, grasslands, boreal and tropical forests, permafrost areas, ocean, etc.) all over the world (reviewed by Reed et al. 2011).

The anaerobic ammonium-oxidising (ANAMMOX) bacteria oxidise ammonium to N_2 using nitrite as an electron acceptor via intermediates, including toxic hydrazine (Mulder et al. 1995). A subunit of the enzyme hydrazine synthase, encoded by *hzsA* gene, is presently thought to be unique to ANAMMOX metabolism and catalyses the synthesis of hydrazine from nitric oxide and ammonium (Harhangi et al. 2012). All known ANAMMOX bacteria identified thus far belong to the order *Brocadiales* in the phylum *Planctomycetes* (Jetten et al. 2010). These organisms are strict anaerobes, but the process is not always inhibited at low oxygen concentrations as was shown by oxygen manipulation experiments in seawater (Jensen et al. 2008). Although some evidence of this process is found also in the upper layer of peat (Hu et al. 2011a), the ANAMMOX has a minor importance in soils and the process is

more common in bioreactors, wastewater plants and landfills (Butterbach-Bahl et al. 2011, Hu et al. 2011b).

The different nitrogen cycling processes are closely coupled with each other. Many microorganisms can conduct multiple pathways in the nitrogen cycle (Nelson et al. 2016), for example some nitrifiers can conduct denitrification, some denitrifiers can be N_2-fixers and some ANAMMOX bacteria are capable of DNRA, nitrate reduction and nitrite oxidation (Lam & Kuypers 2011). In addition, soil communities with high numbers of prokaryotes able to use one nitrogen pathway also generally support higher numbers of prokaryotes that can use other nitrogen pathways (Nelson et al. 2016).

1.2 Peatland management and greenhouse gas emissions

Peatlands are very vulnerable ecosystems to climate change and anthropogenic impacts, and the area of peatlands has been decreasing rapidly over the last century. These ecosystems have been exploited for several economic purposes, where most of them require drainage to lower the water table level for further use as agricultural and forest lands as well as for peat extraction or human settlement purposes. The drainage ditch network across the peatland regulates the soil oxygen and water conditions in order to achieve the best conditions for the cultivated crops, forest or heavy peat harvesting machinery. Land use practices directly affect the distribution and supply of nutrients such as carbon and nitrogen in peat, while management also alters the microbial community abundance and composition in peatland soils (Limpens et al. 2008).

Peatland drainage as well as further extraction actions have been shown to change the dynamics of greenhouse gas emissions. Drainage or extraction process of peatlands increases substantially CO_2 emissions through increased microbial respiration, whereas drainage ditches have often anoxic conditions enhancing methanogenic activity and thus increasing CH_4 emissions from the drained area (Waddington et al. 2009). In addition, studies have shown that the changed water regime considerably increases N_2O emissions from drained soils (Martikainen et al. 1993).

Wetlands, including peatlands, can also be applied as a cost-effective treatment solution for purification of polluted water in distant areas (Kadlec & Wallace 2009). In these kinds of systems, phytoremediation has an important role to treat different types of contaminants, whereas technology is based on the combined action of plants and their associated microbial communities to remove or control many kinds of pollutants (e.g. organic compounds, metals, excessive nitrogen and phosphorus) (Zhang et al. 2010).

1.2.1 Reclamation of abandoned peat extraction areas

Modern peat mining is conducted using large-scale milling and vacuum removal of recently dried peat along with clearance of the surface vegetation (Alexander et al. 2008). Extracted peat is typically used as an energy source in regions rich in peatlands or as a growing medium and soil conditioner in horticulture (Basiliko et al. 2007, Paal & Leibak 2011, Virtanen & Valpola 2011). Furthermore, peat has proven to be effective sorbent for the capture of a wide range of contaminants including heavy metals (Brown et al. 2000, Ringqvist et al. 2002), organic compounds (Costa et al. 2012), oil products (Suni et al. 2004) and excessive phosphorus (Kõiv et al. 2009, Xiong & Mahmood 2010, Kasak et al. 2015) from wastewater and aqueous media. In addition, peat and various peat preparations have been used in the practice of medicine (Beer et al. 2003, Orru et al. 2011) and as compost fabrics, building/insulation material, textile and flavour enhancer (Joosten & Clarke 2002). Due to the wide range of use, peat is an important resource and is being extracted widely, which inevitably leads to the problem of abandoned peat extraction areas.

Once abandoned, cutover peatlands expose well-decomposed peat at the surface with extremely harsh environmental conditions such as altered hydrology, wind erosion, frost heaving and variable physicochemical properties (Huotari et al. 2007), where the reduced microbial activity limits nutrient replenishment (Andersen et al. 2006). Consequently, plant colonisation and growth is also hindered (Huotari et al. 2007). Thus several environmental issues emerge including negative impact on the surrounding hydrologic system, risk of fires, biological and landscape diversity loss (Paal 2011) and continuous greenhouse gas (CO_2, CH_4 and N_2O) emissions into the atmosphere (Andersen et al. 2013). Therefore, it is necessary to restore abandoned peatlands as close as possible back to their natural state (Andersen et al. 2013) or use them in the field of environmental technology such as a bioenergy production sites (Heinsoo et al. 2011, Mander et al. 2012), agricultural lands and berry cultivation sites (Albert et al. 2011), afforestation lands (Caisse et al. 2008), treatment sites of drainage effluent (Paal 2011) and cutaway lakes (Higgins & Colleran 2006, Klavins et al. 2010).

The application of abandoned peat extraction areas to produce biomass for energy purposes is an advisable land-use practice from the perspective of atmospheric impact (Järveoja et al. 2012, Mander et al. 2012). One possible candidate for this application is reed canary grass (*Phalaris arundinacea* L.). The modest growing requirements (low temperature, high moisture, humus richness, drought and flooding tolerance), rapid growth and good burning characteristics of this plant are making it suitable for bioenergy crop in boreal region (Heinsoo et al. 2011, Ghica et al. 2012). Additionally, cultivation cost of reed canary grass is low and the produced biomass can be used for burning as well as for other bioenergy purposes such as for fermentation in bioreactors or as raw material for liquid biofuel or biogas (Ghica et al. 2012).

1.2.2 Treatment wetlands

Treatment wetlands (TW) can be classified as natural treatment wetlands and constructed treatment wetlands. These wetlands include various types of systems, where polluted water flows either horizontally or vertically depending on the structural configuration (Fonder & Headley 2013, Weber 2016).

Natural treatment wetlands are in some cases used as receiving bodies for polluted waters and often provide important treatment functions, whereas these systems were not intentionally designed or modified for a pollution control function (Fonder & Headley 2013). Peatlands are applied to purify runoff from peat extraction areas, arable land and peatland forestry (Nieminen et al. 2014, Wahlroos et al. 2015, Karjalainen et al. 2016). Furthermore, natural treatment wetlands are also used to treat sewage water (Ronkanen & Kløve 2009) and industrial process water such as mining effluent (Räisänen et al. 2001, Palmer et al. 2015).

Widely acknowledged constructed treatment wetlands are artificially created wetland systems designed to enhance and optimise certain physical and/or biogeochemical processes that occur in natural wetlands, with the primary purpose of removing contaminants from polluted waters (Fonder & Headley 2013). These engineered systems allow for a high degree of flexibility in design, while different filter materials (natural filter materials, industrial products and industrial by-products) can be used for treating different types of wastewater (e.g. municipal, domestic, industrial and agricultural wastewaters) (Vymazal 2011, Vymazal 2014, Vymazal & Březinová 2015, Kasak et al. 2016). In constructed wetland systems for the treatment of secondary effluent, peat has been used as a carbon source for denitrifying bacteria that can remove excessive nitrogen (Xiong et al. 2011, Kasak et al. 2015).

Vegetated treatment wetlands have higher treatment efficiency in comparison to the treatment systems without vegetation, but the efficiency is dependent on plant species (Zhang et al. 2010, Vymazal 2013). The presence of macrophytic vegetation has several physical benefits (e.g. filtering and velocity reduction), they conduct the uptake of nutrients and evapotranspiration, create microclimatic conditions, etc. (Zhang et al. 2010, Shelef et al. 2013). In addition, rhizosphere provides a very important base for microorganisms (e.g. releasing gas and exudates) (Berg & Smalla 2009, Zhang et al. 2010, Shelef et al. 2013).

Emissions of greenhouse gases such as CH_4 and N_2O are by-products of treatment wetlands. Several environmental factors controlling greenhouse gas emissions include the availability of carbon and nutrients (especially nitrogen) which directly depend on wastewater loading, temperature, hydrological regime (pulsing vs steady-state flow), groundwater depth, moisture of the filter material and the presence of aerenchyma plants (Hiraishi et al. 2014).

2. THE AIM OF THE STUDY

The general aim of this dissertation was to analyse the effect of different management practices on peatland microbiome and greenhouse gas emissions.

The specific objectives were:

- to explore the factors including management practices (drainage and restoration) that control CH_4 emission in northern peatlands;
- to assess the effect of drainage on the community structure of soil prokaryotes and their genetic potential to perform different nitrogen transformation processes as well as N_2O emission in a tropical peatland;
- to evaluate the effect of reed canary grass cultivation and fertilisation on the prokaryotic community abundance and genetic potential of methanogenesis in residual peat on an abandoned peat extraction area in boreal region, and link these changes to CH_4 emission from the peat;
- to examine the potential of peatlands in wastewater treatment.

3. MATERIAL AND METHODS

The current PhD thesis consists of two original case studies (Paper I and III) and two literature reviews (Paper II and IV). A two-year experiment was conducted on an abandoned peat extraction area in boreal region to analyse the effect of energy crop production on the prokaryotic community structure and its genetic potential for CH_4 production (Paper I). A meta-analysis of the data available in literature was performed to analyse the impact of wetland management on CH_4 emissions in northern peatlands (Paper II). The effect of peat drainage on soil prokaryotic community and microbially mediated nitrogen cycling processes was studied in a tropical peatland (Paper III). An overview about phytoremediation as a promising tool for removal of pollutants from different wastewaters (Paper IV) and additionally the potential of peatland application for these purposes is discussed in this thesis.

3.1 Descriptions of the case studies sites

3.1.1 The abandoned peat extraction area in boreal region (Paper I)

The study area was located in Lavassaare (58°34′20″N, 24°23′15″E) in the largest Estonian peat extraction area (19,746 ha) situated in the Baltic region of Northern Europe (Figure 2). The region has a temperate climate with a 30-year (1981–2010) mean annual temperature of 6.3°C and annual precipitation of 746 mm (Estonian Weather Service). The systematic industrialised extraction of peat for commercial purposes dates back to the mid-twentieth century and lasted until 2006 in this particular study area. The thickness of residual peat column was variable in depth (0.3–1.2 m) across the area and consisted primarily of well mineralised *Phragmites-Carex* peat (H7 class according to the von Post decomposition scale). The area was divided into 20 m wide strips by the drainage ditches. Some of the abandoned peat extraction strips were sowed with reed canary grass (*Phalaris arundinacea* L. Estonian-bred variety "Pedja") in the spring of 2007.

In 2012, three cultivated and three uncultivated peat strips were chosen for the experiment. On each strip, one fertilised and one control plot (2.5 x 10 m) with 0.5–0.7 m peat layer were set up. All the plots were located at 4 m distance from the ditches. The distance between control and fertilised plot was at least 4 m on a peat strip. Based on the chemical analysis of the study site peat, mineral fertiliser containing nitrogen (72 kg), phosphorus (18 kg) and potassium (36 kg) was applied per hectare once per year (in June) on fertilised plots.
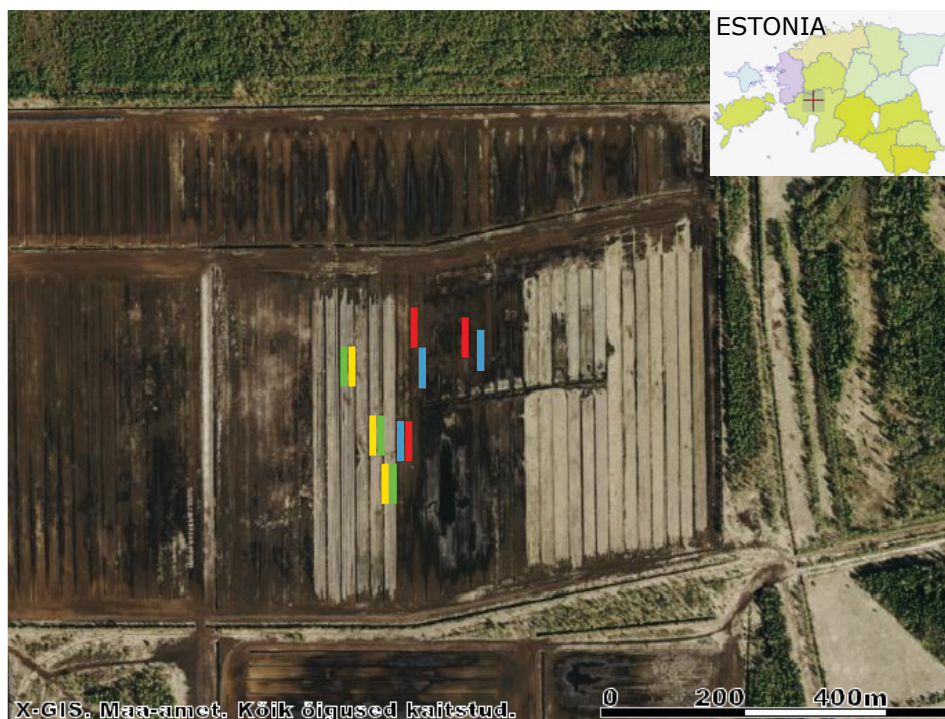
Figure 2. Lavassaare study area with the experimental plots: cultivated plots (control (green) and fertilised (yellow)); uncultivated plots (control (blue) and fertilised (red)).

3.1.2 Tropical peatland sites (Paper III)

The studied tropical peatland is situated in the north-eastern part of French Guiana, where the average monthly temperature is around 26°C showing minor variation between seasons and average annual rainfall is between 3000–4000 mm (Cubizolle et al. 2013). The natural peatland site was located close to the village of Tonate (4°59'27"N, 52°27'14"W) and drained site near the town of Kourou (5°09'42"N, 52°39'06"W) (Figure 3). At both study sites, peat layer was approximately 0.3–0.4 m thick and the dominating plant species was *Eleocharis interstincta* with approximate coverage of 99%. Nine sampling points (with a distance of 25 m between points) were established at 2,500 m^2 area on both study sites. On the drained site, the three first sampling points were located at 50 m distance from a 2–3 m deep drainage ditch.
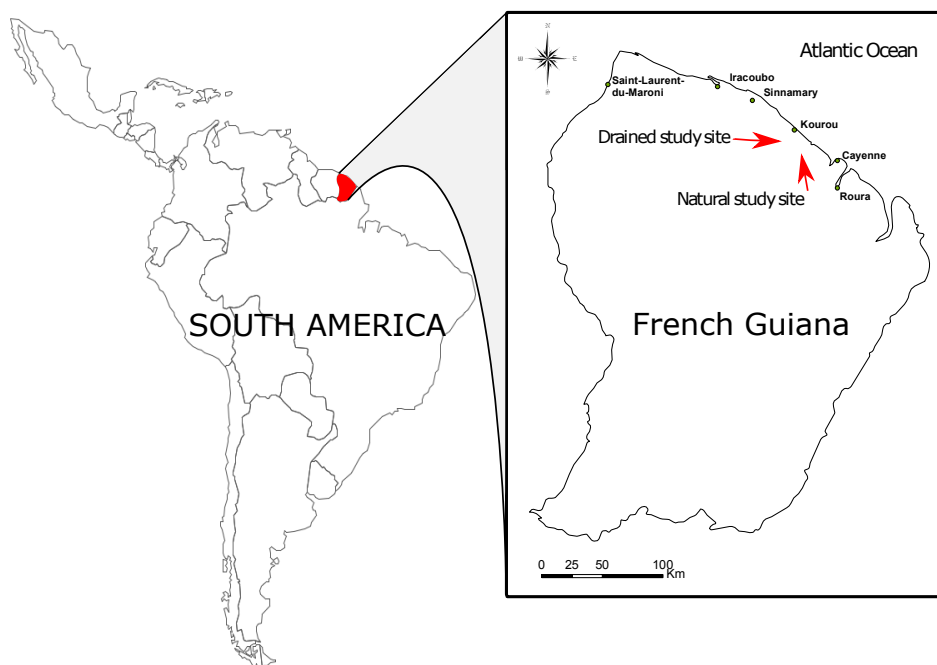
Figure 3. Locations of the study sites in French Guiana.

3.2 Sampling of peat and gases

A total of 216 composite peat samples (from the depths of 0–20, 20–40 and 40–60 cm) were collected during three sampling campaigns (June 2012, September 2012 and September 2014) from the Lavassaare peat extraction area (Figure 4). The in situ CH_4 emission measurements were conducted using closed chamber technique (Hutchinson & Livingston 1993); soil temperature and water table depth were also regularly measured on each plot during the experiment. The sampling is described in detail in Paper I.
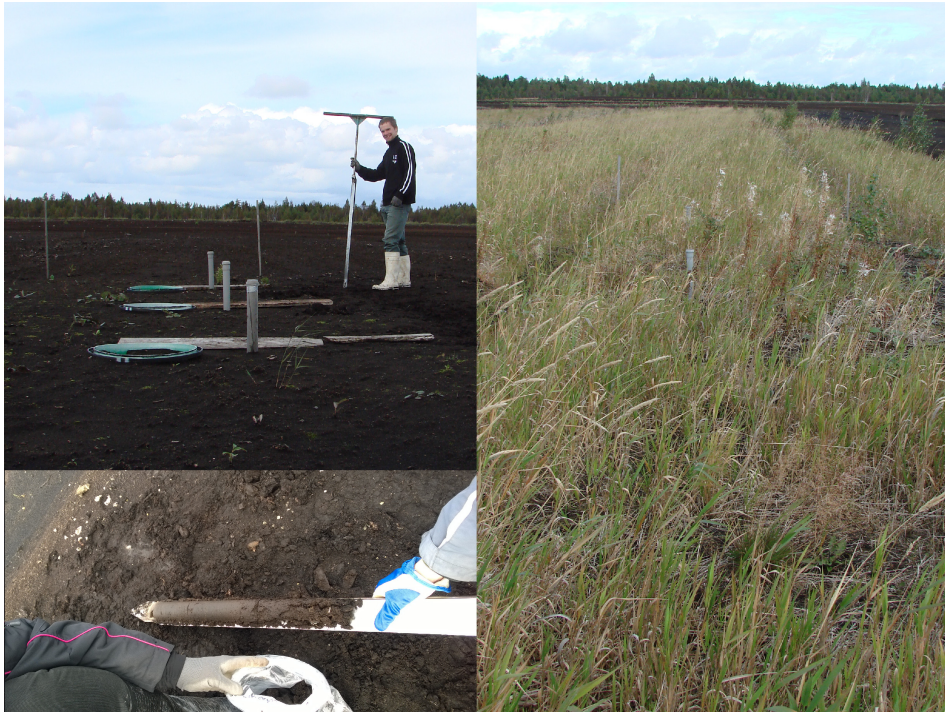
Figure 4. Uncultivated (left) and *Phalaris* cultivated (right) sampling plots in Lavassaare experimental area (Estonia) and peat profile taken during the sampling from an uncultivated plot (bottom).

In French Guiana, 18 composite samples (nine samples from natural and nine from drained sites) from the 0–10 cm soil layer were collected in October 2013 (Figure 5). In addition, intact soil cores were taken into cylinders from the top layer of soil (0–10 cm) from each French Guiana sampling point to evaluate potential N_2 emission from soil using helium atmosphere soil incubation technique in laboratory. During the peat sampling, in situ N_2O and CH_4 emission measurements were conducted using the closed chamber technique (Figure 5) (Hutchinson & Livingston 1993) and soil temperature and water table depth were determined at each sampling site as is described in detail in Paper III.
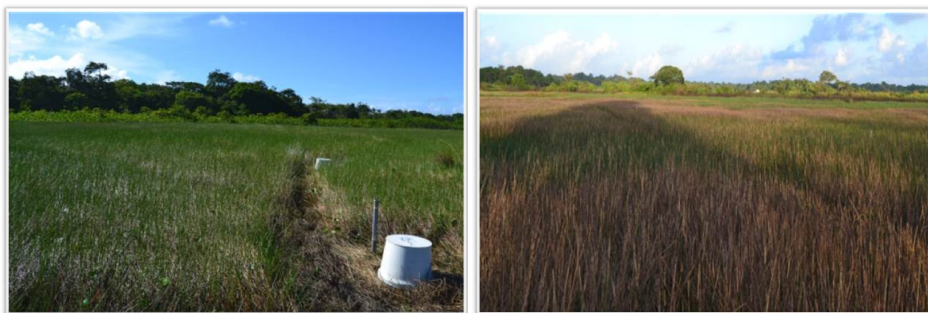
Figure 5. Natural (left) and drained (right) sampling sites in French Guiana. Closed gas measurement chambers can be seen on the natural site.

Peat samples were stored at cooled conditions during the transportation. In the laboratory, all collected peat samples were homogenised and divided into subsamples for chemical and molecular analyses. Sub-samples for molecular analyses were stored at $-20°C$ and for chemical analyses at $+4°C$.

3.3 Chemical analyses of peat samples

Chemical analyses were conducted shortly after sampling from collected peat samples (Paper I and III). The following parameters were measured using standard methods during both case studies: pH, Kjeldahl nitrogen (N), ammonium nitrogen (NH_4-N), nitrate (NO_3-N), total phosphorus (P), calcium (Ca) and total potassium (K) concentrations. Additionally, dissolved organic carbon (DOC), phosphate (PO_4-P), total sulphur (TS) and sulphate (SO_4-S) contents were determined for Lavassaare soils and the soil water content, total carbon (C) and magnesium (Mg) contents in French Guiana soil samples were measured.

3.4 Microbiological analyses of peat samples

3.4.1 DNA extraction and quantitative PCR

In the case of all peat samples, the DNA was extracted using PowerSoil DNA Isolation kit (MO BIO Laboratories Inc., CA, USA) according the manufacturer`s instructions (Papers I and III). Homogenisation of samples was performed using Precellys® 24 (Bertin Technologies, France) at 5000 rpm for 20 s. The quality and quantity of extracted DNA were determined with spectrophotometry Infinite M200 (Tecan AG, Austria). The extracted DNA was stored at $-20°C$ prior to further analyses.

Quantitative PCR (qPCR) was applied to evaluate the bacterial and archaeal community size by quantifying the abundance of bacterial and archaeal specific

16S rRNA genes, respectively (Papers I and III). The genetic potential of CH_4 production (Paper I) and nitrogen transformation processes (Paper III) were evaluated by targeting the following functional genes: *mcrA* (methanogenesis); *nirS*, *nirK*, *nosZ* clade I and *nosZ* clade II (denitrification); *nifH* (N_2 fixation); *nrfA* (DNRA); bacterial and archaeal *amoA* (nitrification); comammox *amoA* clade A and clade B (complete ammonia oxidation) and ANAMMOX-specific 16S rRNA genes (anaerobic ammonium oxidation). New primer sets were designed for the amplification of archaeal 16S rRNA gene and *mcrA* gene fragments (Paper I).

All qPCR amplifications were performed with RotorGene® Q (QIAGEN, CA, USA) in a 10 µl reaction mixture containing 5 µl Maxima SYBR Green Master Mix (Thermo Fisher Scientific Inc., MA, USA) and the optimised concentrations of primers for each primer set, 1 µl template DNA and sterile distilled water. The detailed descriptions of the used qPCR reactions conditions, primer concentrations and amplification programs are described in Papers I and III.

The concentrations of target gene abundances in the soil samples were presented as gene copy numbers per gram of dry soil weight. Additionally, the proportions of different functional genes were calculated as relative abundances from the abundance of bacteria and archaea. The detailed description of the calculation method is described in Papers I and III.

3.4.2 Preparation of DNA libraries for metagenomics analysis, sequencing and data processing

The soil microbial community in tropical peatland was profiled using the Illumina NextSeq 500 sequencing system (Illumina, CA, USA). A detailed description of the PCR reactions, pooling of amplicons, DNA library preparation and sequence analyses is given in Publication III.

About 1% of paired-end reads had low quality caused by sequencing errors and after denoising step, 9–14 million reads per sample were obtained. Kaiju v 1.4.5 was used to classify metagenomic reads down to the species level (Menzel et al. 2016). To screen the metagenomes for the potential function, existing databases of marker genes (amino acid sequences) were used as a reference: the *nirK* gene (Decleyre et al. 2016); the *nirS* and *nosZ* genes (Graf et al. 2014); the *nifH*, *nrfA*, *hzsA*, bacterial *amoA* and *pmoA* and archaeal *amoA* genes (Lüke et al. 2016). According to these references, edge principal components analysis (edge PCA) was performed and graphics were made with the R package ggplot2 v 2.1.0 (Wickham 2009) and the Archaeopteryx tree viewer v 0.9920 (Han & Zmasek 2009). The detailed description of the methods used is described in Publication III.

3.5 Meta-analyses of methane emissions from northern peatlands (Paper II)

The meta-analysis included a total of 87 studies reporting measurements of CH_4 emissions taken from 186 sites covering different countries (Finland, Sweden, Estonia, UK, Canada, USA, etc.), peatland types (fen, bog, wooded fen and bog) and management systems (drainage, peat extraction, restored, etc.) to evaluate the impacts of management on CH_4 emissions from northern (latitude 40° to 70°N) peatlands. To discover all papers that have reported CH_4 emissions from northern peatlands, a comprehensive search was performed on the Web of Science database (accessed between January 2013 and July 2016) using the keywords: pristine peatlands, CH_4 emissions, drainage, restoration, fens, bogs, mire and northern peatlands. To gain widespread coverage, all references in the papers found in the Web of Science search were also checked. Only studies which covered at least one growing season and had weekly or more frequent measurement intervals were selected. The detailed description of the methods used for the analyses can be found in Paper II.

3.6 An overview of treatment wetlands (Paper IV)

Results from 132 published studies were integrated together to review the basic processes of phytoremediation with special emphasis on rhizoremediation and plant-microbe interactions in plant-assisted biotransformation of organic and inorganic pollutants in soil and treatment wetlands (Paper IV). 33 successful studies of phytoremediation of various organic contaminants using rhizospheric or endophytic bacteria were presented and many studies of treatment wetlands treating municipal or industrial wastewater were described. The results from publications concerning particularly the potential of peatlands in wastewater treatment are discussed in this thesis.

3.7 Statistical analyses

In all tests, statistical significance was determined at a 95% confidence level. One-way ANOVA and Tukey HSD post hoc tests and t-tests were applied to evaluate the significance of the differences between soil groups (Papers I and II). Additionally, linear mixed-effects model (LMM) was applied to enable simultaneous consideration of all the factors that potentially contribute to the understanding of the structure of the studied parameters in Papers I and II. To evaluate the significance of the differences between study sites in physico-chemical variables, gene parameters, phylogenetic data and emission values, multivariate linear models were constructed and models were tested using the anova() function (Paper III). Spearman's Rank correlation coefficients were calculated to determine significant relationships between different gene and

environmental parameters (Papers I and III). Interpolated contour plots were created for pairs of environmental parameters as x and y with annual CH_4 emissions as the z variable (Paper II).

Principal component analyses (PCA) were performed on the soil physicochemical and microbiological data (gene copy numbers and proportions of bacterial and archaeal genera) (Paper III). Between-class analysis (BCA) was applied to find the principal components based on the centre of gravity of log-transformed values of soil chemical parameters using a single factor (sampling time, soil layer, cultivation or cultivation and fertilisation) as instrumental variable (Paper I). Edge principal components analysis (edge PCA) was performed to detect important differences between natural and drained meta-genomic samples that contain closely related taxa (Paper III).

The significance of the differences between analysed soil groups was tested using a Monte-Carlo permutation test (9,999 permutations) in case of BCA in Paper I. In Paper III, the difference in microbial community structure in cases of PCA and edge PCA between sites was evaluated using permutational multi-variate ANOVA (PERMANOVA) with 9999 permutations. The ordination results of different edge PCAs were compared with a Procrustes rotation and 9999 permutations to assess pairwise marker gene community structures concordance (Paper III).

Distance-based regression analysis was applied with forward selection procedure and 9999 permutations to identify soil physicochemical variables that explain significant amounts of variation in marker gene community structure (Paper III).

Main tool for calculations and statistics was software R (Papers I–III).

4. RESULTS AND DISCUSSION

4.1 Drainage of northern and tropical peatlands

Natural northern peatlands (pristine) are important sources of CH_4 emission with an average annual flux of 12 ± 21 g C m^{-2} year^{-1} (median is 4.3 g C m^{-2} year^{-1}) to the atmosphere considering all sites, vegetation types and locations studied in Paper II. Emissions between the sites were highly variable with a 95% confidence interval of 7.6–15.7 g C m^{-2} year^{-1} for the mean and 3.3–6.3 g C m^{-2} year^{-1} for the median. CH_4 emissions from the fens (mean 15.4 g C m^{-2} year^{-1}) were significantly higher than those from the bogs (mean 7.1 g C m^{-2} year^{-1}). Bogs and fens differ in abiotic and biotic factors. These abiotic and biotic differences lead to the fens having higher methanogenic activity (Juottonen et al. 2005), higher litter degradation rate (Aerts et al. 1999), and thereby higher CH_4 emissions (Nykänen et al. 1998), compared to the bogs. The correlation between CH_4 flux and water table depth was significant for the fens but not for the bogs or wooded fens and bogs (Paper II). The optimal water table depth for CH_4 production was consistently below the peat surface in the bogs and near to the peat surface for the fens. The CH_4 flux and water table depth were significantly correlated in the studied peatlands and the following relationship was revealed between these factors: $CH_4 = 32.462 \times exp^{(0.08 \times WT)} (n = 87, r^2 = 0.54, p < 0.01)$, where WT is the water table depth.

In addition, a trend toward higher CH_4 emissions from natural northern peatlands was shown with higher water table and higher pH and lower temperature (peaking at mean annual air temperature of approximately 2°C). Peatland type, pH, water table and air temperature are statistically important factors in determining CH_4 flux in natural peatlands (Paper II). Many studies have reported the influence of water table depth (Granberg et al. 1997, Frenzel & Karofeld 2000, Moore & Dalva 2006, Yang et al. 2006), pH (Hutsch 1998, Singh et al. 1999) and temperature (Granberg et al. 1997, Saarnio et al. 1998, Ding & Cai 2007) on CH_4 emissions from different peatlands.

Drainage of natural peatlands changes initial soil biogeochemical processes and the balance of greenhouse gases (CO_2, CH_4 and N_2O) in different climate zones. Drainage of water-saturated peat soils will improve aeration leading to lower CH_4 emissions, although it may result in considerable losses of carbon and nitrogen to the atmosphere through CO_2 and N_2O emissions (Oleszczuk et al. 2008).

The drainage practices in northern peatlands clearly reduce the CH_4 flux, on average, by 84% compared to the original emission values with a mean of 8.3 g C m^{-2} year^{-1} (Paper II). This effect is similar for all types of drained peatlands regardless of land use and vegetation cover. Still, drainage ditches themselves can become new anaerobic zones, with similar characteristics to the undrained peat and with similar or even increased CH_4 emissions (Sundh et al. 2000, Huttunen et al. 2003, Schrier-Uijl et al. 2010). These higher emissions from drainage ditches could be large enough to compensate the reduced CH_4

emissions from the drained peatland area (Minkkinen et al. 2008). Furthermore, drainage influences CH_4 emissions from fens more than from bogs (Paper II). Maljanen et al. (2010) also showed that fens are more sensitive to the drainage (water table depth) compared to bogs. Yrjälä et al. (2011) found that several years of drying of northern peatland changed the structure of the plant community and also soil microbial communities that control greenhouse gas emissions.

Results of this study show that drainage also changed gaseous emissions of the studied tropical peatland (Paper III; Figure 6). The average CH_4 flux from the natural site was significantly ($p<0.001$) higher than that for the drained site. The average N_2O flux from the natural site was significantly lower than that for the drained site. The potential N_2 emission was highly variable from the top layer of soils (0–10 cm) of both study sites and the potential was significantly greater at the natural site. Furthermore, results of this study show that the drainage also changed microbial community structure of the studied tropical peatland. The total bacterial abundance decreased and archaeal abundance increased in response to the drainage; the archaeal abundance exceeded the bacterial abundance by more than one order of magnitude in the drained site. These two groups were almost equally represented in the community at the natural site.

Proteobacteria, *Actinobacteria*, *Acidobacteria* and *Firmicutes* were the dominant bacterial phyla at both study sites of the tropical peatland (Paper III). Overall, sequences belonging to 32 different bacterial phyla were identified from the studied soils but the composition of the bacterial phyla differed between the natural and drained sites. *Proteobacteria* was the most abundant phylum (40% of the total effective bacterial sequences) at the natural site, followed by *Actinobacteria* (21%), *Acidobacteria* (13%), *Firmicutes* (7%) and *Planctomycetes* (4%). The most abundant bacterial phyla at the drained site were *Actinobacteria* (38%), *Proteobacteria* (34%), *Firmicutes* (8%) and *Acidobacteria* (4%). Only 1% of the sequences from the drained site belonged to the phylum *Planctomycetes*. These results are mainly in line with a meta-analysis study which also found similar trends in the changes of bacterial phyla proportions after the conversion of natural tropical soils into agricultural soils (Trivedi et al. 2016). The only exception was *Planctomycetes* group which proportion showed an opposite effect compared to the results of this study (its proportion increased due to the agriculture). At the genus level, there was some correspondence in dominant genera between the natural and drained sites (*Mycobacterium* and *Conexibacter* from phylum *Actinobacteria*; *Burkholderia*, *Rhodoplanes* and *Pseudomonas* from the phylum *Proteobacteria*; *Paenibacillus* from the phylum *Firmicutes*); however, the natural soils were mainly driven by the dominant genera "*Candidatus* Koribacter", "*Candidatus* Solibacter", *Granulicella*, *Acidobacterium* and *Terriglobus* from the phylum *Acidobacteria*; *Singulisphaera* and *Planctomyces* from the phylum *Planctomycetes*; *Bradyrhizobium*, *Geobacter* and *Methylobacterium* from the phylum *Proteobacteria*. By contrast, *Streptomyces*, *Frankia*, *Rhodococcus*, *Pseudonocardia*,

Amycolatopsis, *Actinoplanes*, *Streptosporangium*, *Thermomonospora* and *Catenulispora* from phylum *Actinobacteria* were abundant genera in the drained soils.

The results revealed the importance of archaea, especially in drained tropical peatland (Paper III). The dominant archaeal phyla at both study sites were *Euryarchaeota*, *Crenarchaeota* (class *Thermoprotei*), *Thaumarchaeota* and *Korarchaeota*. Differences in the proportions of *Euryarchaeota* and *Crenarchaeota* between the natural and drained site were significant. At the natural site, more than half of the *Euryarchaeota* belonged to the class *Methanomicrobia*, whereas *Methanomicrobia* and *Thermoplasmata* were the most abundant euryarchaeal classes at the drained site. The results indicated that CH_4 production was more regulated by methanogens from euryarchaeal class *Methanomicrobia* at the natural sites, compared to the drained sites, whereas methanogenic archaea from class *Thermoplasmata* appeared to be also important at the drained sites; however, methanogens from class *Methanobacteria* were similarly represented at both sites. This result is consistent with a study of microbial community composition in an upper layer of peat (0–30 cm) of pristine and drained boreal ecosystems (i.e. bog, fen, spruce swamp forest), where percentages of *Methanomicrobia* and *Thermoplasmata* were also reduced and increased, respectively, after long-term drainage (Urbanová & Bárta 2016). In addition to bacteria, N_2 fixation is shown to be widespread among methanogenic *Euryarchaeota* (Cabello et al. 2004), which were significantly more abundant at the natural site. Many archaeal genera were differentiated between the natural and drained sites. For example, "*Candidatus* Nitrosotenuis", *Nitrosopumilus* and "*Candidatus* Nitrosopelagicus" from the phylum *Thaumarchaeota*; *Thermococcus*, *Methanobacterium*, *Pyrococcus*, *Methanobrevibacter*, *Methanocaldococcus*, *Geoglobus*, *Methanothermobacter*, *Ferroglobus* and *Halobacterium* from the phylum *Euryarchaeota* were among the taxa most represented in all the investigated soils, while *Methanocella*, *Methanosarcina*, *Methanosaeta*, *Methanoregula*, *Methanoculleus*, *Methanosphaerula*, *Methanospirillum*, *Methanococcoides*, *Methanococcus*, *Haloferax* and *Methanolobus* from the phylum *Euryarchaeota* were particularly more abundant in the natural soils than in their drained counterparts. By contrast, *Sulfolobus*, *Thermofilum*, *Vulcanisaeta*, *Thermoproteus*, *Metallosphaera*, *Caldivirga*, *Caldisphaera* and *Acidilobus* from the phylum *Crenarchaeota*; *Archaeoglobus*, *Aciduliprofundum*, *Thermoplasma*, *Methanomassiliicoccus*, *Ferroplasma*, *Picrophilus* and "*Candidatus* Methanoplasma" from the phylum *Euryarchaeota*; "*Candidatus* Korarchaeum" from the phylum *Korarchaeota* were more abundant genera in the drained soils.

Natural

N-FIXATION

nifH
(4.58 ± 0.79%)

Slackia
Methanopyrus
Desulfitobacterium

N_2
12.9 ± 5.5
g C m^{-2} year^{-1}

N_2O
0.003 ± 0.015
g C m^{-2} year^{-1}

CH_4
35.8 ± 24.5
g C m^{-2} year^{-1}

NITRIFICATION

amoA archaea
(0.15 ± 0.07%)

Thaumarchaeote
Nitrosopumilus

DNRA

nrfA
(0.00005 ± 0.00005%)

Anaeromyxobacter
Myxococcus
Wolinella
Desulfovibrio
Geobacter

DENITRIFICATION

nirK
(1.25 ± 0.19%)

Gemmatimonas
Opitutus
Sulfobacillus

nirS
(0.011 ± 0.003%)

Pseudogulbenkiania
Cupriavidus
Rubrivivax

nosZI
(0.16 ± 0.03%)

Rhodanobacter

nosZII
(0.05 ± 0.01%)

diverse community

Drained

N_2
7.1 ± 3.8
g C m^{-2} year^{-1}

N_2O
0.09 ± 0.10
g C m^{-2} year^{-1}

CH_4
0.05 ± 0.07
g C m^{-2} year^{-1}

N-FIXATION

nifH
(0.39 ± 0.12%)

diverse community

NITRIFICATION

amoA archaea
(0.22 ± 0.09%)

Thaumarchaeote

DENITRIFICATION

nirK
(0.58 ± 0.14%)

Methylocella
Methylotenera
Paraburkholderia
Burkholderia
Ralstonia

nirS
(0.00004 ± 0.00002%)

Nitratifractor
Sulfurovum
Sulfurimonas
Pyrobaculum

nosZI
(0.03 ± 0.02%)

diverse community

nosZII
(0.007 ± 0.006%)

Desulfomonile
Anaeromyxobacter
Opitutus
Diplosphaera

Figure 6. The influence of drainage on functional gene proportions in prokaryotic communities and diversity of nitrogen-transforming microbial groups in the natural and drained tropical soils. The most abundant microbial groups are shown based on edge PCA. The gene proportions and fluxes of gases (N_2, N_2O and CH_4) are described by their means and standard deviations.

Drainage had a significant effect on the abundance of nitrogen-transforming microbial community (Paper III). *nirS*, *nirK*, *nosZI*, *nosZII*, *nifH* and archaeal *amoA* genes were detected in all samples of both tropical study sites, whereas *nrfA* genes were detected only from the natural site. The abundances of *nirS*, *nosZI*, *nosZII* and *nifH* were significantly higher in the natural site soil, and the archaeal *amoA* abundance was higher in the drained soil. Bacterial *amoA* and ANAMMOX-specific 16S rRNA genes were not detected from either of the study sites. These results were also confirmed by metagenomic analysis, in which neither *hzsA* (ANAMMOX) nor bacterial *amoA* genes were detected in the study site samples, and *nrfA* genes were not detected in samples from the drained site. The proportions of *nirS*, *nirK*, *nosZI*, *nosZII* and *nifH* in prokaryotic communities were significantly higher in the natural site soil than in the drained site soil, whereas the proportions of archaeal *amoA* appeared to be fairly similar at the natural and drained sites (Figure 6). The ratios between *nirS* and *nirK* as well as *nosZ* and *nir* genes were significantly higher at the natural sites than at the drained sites, whereas the ratio of *nosZI* and *nosZII* was not significantly different between the two sites. The balance between *nosZ* and *nir* genes was in favour of the latter genes at both study sites. Molecular studies have shown that the abundance of *nir* genes usually significantly exceeds *nosZ* abundance in various environments (Jones et al. 2013, Ligi et al. 2014). Quantification of comammox bacterium ("*Candidatus* Nitrospira inopinata") was not successful when using the only available comammox *Nitrospira* specific primer pair due to unspecific amplification, although metagenomic analysis revealed the presence of this bacterium (sequence abundance 0.14–0.19% and 0.09–0.13% of all classified bacteria at the natural and drained sites, respectively).

Drainage also had a significant effect on the structure of nitrogen-transforming microbial community (Paper III). The edge PCA results indicated significant differences between natural and drained sites for *nirS*, *nirK*, *nifH*, archaeal *amoA* and all *nosZ* gene phylogenetic diversity (Figure 6). In all cases, the first principal component from the edge PCAs provided main separation of the samples. For *nirK*-harbouring microorganisms, the first principal component of edge PCA was related to the higher relative abundance of the genera *Gemmatimonas*, *Opitutus* and *Sulfobacillus* at the natural site, while the genera *Methylocella*, *Methylotenera*, *Paraburkholderia*, *Burkholderia* and *Ralstonia* were more abundant at the drained site. The diversity of *nirS*-harbouring microorganisms varied along the first PCA axis for the drained site. The difference in *nirS*-harbouring microbes between the natural and drained soils was primarily due to the higher contribution of the genera *Pseudogulbenkiania*, *Cupriavidus* and *Rubrivivax* at the natural site and of *Nitratifractor*, *Sulfurovum*, *Sulfuri-monas* and *Pyrobaculum* at the drained site. According to Graf et al. (2014), the majority of species of the aforementioned *nirS*-type denitrifiers genera possess *nosZ* gene, although only half of the species of the aforementioned *nirK*-type denitrifiers genera possess *nosZ* gene. A superposition of the *nosZI*-harbouring microbial community composition on the phylogenetic tree showed that the drained site had a diverse and heterogeneous community, while only the *nosZI*

gene-possessing genus *Rhodanobacter* was abundant at the natural site. The genus *Rhodanobacter* has been shown to be an important group of denitrifiers in acidic soils (van den Heuvel et al. 2010). In contrast, the natural site possessed a diverse community of *nosZII*-harbouring microbes, while only four genera (*Desulfomonile*, *Anaeromyxobacter*, *Opitutus* and *Diplosphaera*) were detectable at the drained site. The edge PCA of *nifH*-harbouring micro-organisms indicated that the natural site had a higher abundance of *Slackia*, *Methanopyrus* and *Desulfitobacterium*, while the drained soil had a more diverse set of genera. The separation of *amoA*-harbouring archaea between the natural and drained sites occurred primarily along the edge PCA first principal component and was related to the higher abundance of *Nitrosopumilus* at the natural site; other differences were attributed to an uncultured *Thaumarchaeota*. For *nrfA* gene-possessing microbes, the edge PCA showed their presence only at the natural site (genera in decreasing order of occurrence: *Anaeromyxobacter*, *Myxococcus*, *Wolinella*, *Desulfovibrio*, *Geobacter*, *Desulfitobacterium*, *Dethio-bacter*, *Carboxydothermus* and *Slackia*). As was indicated by the Procrustes analysis, similar patterns for community differences across the sites were observed for *nirK*, *nirS*, *nosZ*, *nifH* and archaeal *amoA* functional genes.

Relationships between studied gene parameters were quite different when comparing natural and drained tropical peatland sites (Paper III). At the natural site, the archaeal 16S rRNA gene abundance was strongly related to most of the detected nitrogen transformation gene abundances (except *nrfA*), while only two significant correlations were found between gene abundances (archaeal 16S rRNA and *nosZI*; *nifH* and archaeal *amoA*) in the drained soil. The relationships between bacterial 16S rRNA gene abundance and *nosZI* and archaeal *amoA* and the particularly strong relationship with *nifH* abundance were revealed by a correlation analysis of the natural site. For the drained site, strong correlations between the bacterial and archaeal 16S rRNA proportions and the *nifH* and archaeal *amoA* proportions were detected. The *nifH* abundance was found to be related to most of the studied denitrification pathway genes (except *nosZII*) at the natural site, while at the drained site, the proportion of *nifH* was related to *nosZI* and archaeal *amoA* proportion in the prokaryotic community. No relationships were found between *nrfA* and other targeted genes for either of the study sites.

Several statistically significant relationships were found between the studied gene parameters and physicochemical factors, but the patterns of these relationships were not similar for the drained and natural sites (Paper III). The soil carbon and nitrogen contents and their ratio (C/N) were related to the abundances of bacterial and archaeal 16S rRNA genes and their proportions in the total prokaryotic community in the soil, but the effect differed between the two study sites. In addition, the archaeal 16S rRNA gene abundance showed a strong positive correlation with the soil water content in the drained soil. In the natural site soil, the pH had a strong effect on the studied gene abundances (bacterial 16S rRNA gene, *nirS*, *nosZI*, *nifH* and archaeal *amoA*), their pro-portion (*nirK*) and their ratios (*nosZ/nir* and *nosZI/nosZII*), but this was not the

case for the drained site. Soil pH has been shown to be a key factor for controlling the abundance and community composition of AOA (Hatzenpichler 2012, Oton et al. 2015). The soil carbon and nitrogen (or C/N) content was strongly related to the nitrogen transformation gene abundances (*nirS*, *nosZI*, *nifH* and archaeal *amoA*) in only the natural soil, the gene proportions (*nirK*, *nifH* and archaeal *amoA*) in only the drained soil, and the gene ratios (*nosZI/nosZII* and *nosZ/nir*) in the microbial communities for both study sites. The *nrfA* proportion in the natural site prokaryotic community showed correlations with the soil chemical composition (different nitrogen fractions, phosphorus and potassium content), while no correlations were found between this gene and the environmental parameters in the drained soils. The *nosZII* abundance and proportion were related to the phosphorus content in the drained soil. The abundance of *nirS*-type and *nosZI*-type denitrifiers showed a similar pattern in response to the edaphic factors in the natural soil. These results are consistent with those of Stone et al. (2015), who also found that the abundances of *nirS* and *nosZ* were positively correlated with soil carbon, nitrogen and phosphorus concentrations in humid tropical forests in Puerto Rico. Distance-based regression analysis showed that soil chemical variables (especially nitrogen and its soluble inorganic fractions) explained a significant amount of the variation in the community structures of nitrogen-transforming genes. Additionally, the soil water content contributed to the variation of *nifH* gene-possessing microbes in the study soils.

The study found significant relationships between studied gene parameters and nitrogen gas (N_2 and N_2O) emissions for both natural and drained tropical peatland sites (Paper III). The observed correlations suggested that *nirS*-type and *nosZI*-type denitrifiers play an important role in controlling the N_2O and N_2 gas fluxes in the natural peatland soils. Contrary to the natural sites, microbes harbouring *nosZII* gene more likely performed N_2O transformation to N_2 in the studied drained soils. It can be assumed that the discrepancy between the denitrification pathways in these tropical peatland sites might be caused by a selective pressure. This finding is supporting the hypothesis that because of two different N_2O reductase mechanisms, one of those pathways to be preferred over the other in different environmental conditions (Jones et al. 2013). In addition, according to the positive relationship between *nrfA* proportion and N_2O gas emission in the natural sites, it can be inferred that DNRA plays an important role in controlling the N_2O gas fluxes in these natural peatland soils. Templer et al. (2008) reported that the rates of DNRA were accounting for approximately 35% of gross nitrification in a humid tropical forest soil in Puerto Rico and were much higher than the rates of N_2O production from denitrification. Gene ratios (*nirS/nirK* and *nosZ/nir*) showed positive correlations with N_2 emission at the natural site, whereas the ratio *nosZI/nosZII* was negatively correlated to N_2 emission at the drained site. No relationships were found between studied gene parameters and N_2O emission for the drained site.

4.2 Climate-responsible management of peatlands

Northern peatlands, previously drained for many uses (e.g. forestry, cropping, grazing and mining), emit more CH_4 after the restoration (rewetting) compared to the pre-restoration emission (Paper II). Restoration increased CH_4 flux by an average of 1.3 ± 6.5 g C m^{-2} year^{-1} (46%). However, the change in CH_4 flux due to rewetting was not statistically significant with mean flux before restoration being 3.0 ± 3.1 g C m^{-2} year^{-1} and after restoration being 4.2 ± 6.3 g C m^{-2} year^{-1}. This indicates a different response to rewetting between sites, which have different previous management practices and initial peatland type. CH_4 emission could be reduced by creating different vegetation compositions (Komulainen et al. 1998, Tuittila et al. 2000, Waddington & Day 2007) that lead to changes in the peat properties and methanogenic community (Basiliko et al. 2004). Mahmood and Strack (2011) reported a significant correlation between CH_4 emissions and vegetation cover on an abandoned peatland. Vegetation may stimulate CH_4 emissions by providing substrates for gas production and transportation to the atmosphere (Wilson et al. 2013). In Canada and Ireland, CH_4 emissions from restored cutover peatlands increased in the first three years following restoration due to the fresh substrates provided by the new vegetation cover (Waddington & Day 2007, Wilson et al. 2013). The establishment of vascular vegetation following peat extraction is generally more extensive on cutover fens than on cutover bogs (Graf et al. 2008). Although a combined transportation of oxygen with CH_4 by aerenchyma tissues of plants could reduce CH_4 emissions, previous studies reported higher emissions from vascular plants, especially from sedges (Waddington et al. 1996). Roulet et al. (1993) and Roulet and Moore (1995) reported approximately 23–57 times greater CH_4 emissions from restored herbaceous vegetation site than from an herbaceous vegetation cutover site. Moreover, common cotton grass (*Eriophorum vaginatum*) generates large CH_4 fluxes (Greenup et al. 2000) from peat, whereas peat mosses (*Sphagnum* spp) can act as a CH_4 sink (Raghoebarsing et al. 2005) in restored peatland.

The difference in CH_4 emissions was significantly affected by the land use after drainage (cropland, grassland, natural or woodland) (Paper II). Changes with time in CH_4 emissions patterns after rewetting may be related to previous land use. Although restoration increases CH_4 fluxes, it could reduce net greenhouse gas emissions by reducing CO_2 flux (Baird et al. 2009, Samaritani et al. 2011, Beetz et al. 2013, Strack & Zuback 2013). The new emerging plant community and altered hydrology resulting from restoration should lead the dynamics of greenhouse gases more similar to the natural peatlands. The increase in CH_4 emissions due to restoration must be considered when land use strategies to reduce emissions are developed.

Using abandoned peat extraction areas to produce energy crop (e.g. reed canary grass) biomass for energy purposes has been suggested as an option to mitigate atmospheric impact in peatland-rich Northern Europe (Hyvönen et al. 2009, Järveoja et al. 2012, Mander et al. 2012). The response of the soil

microbial abundance to the reclamation of an abandoned peat extraction area with a bioenergy crop determined primarily CH_4 fluxes (Paper I). The CH_4 emission rates were relatively small (-0.04 to 0.25 g C m^{-2} year^{-1}) and in many cases even some consumption of this gaseous compound was detected from the peat of the study plots of this abandoned peat extraction area. The CH_4 emission from uncultivated and cultivated plots was not significantly different. The emission from the plots stayed in the range (-0.23 to 0.58 g C m^{-2} year^{-1}) of those reported previously in different disturbed peatlands: semidry pine-dominated mesotrophic peatland forest (Yrjälä et al. 2011); drained and hay cultivated peatlands (Berglund & Berglund 2011); abandoned peat extraction areas and its different reed canary grass treatments (Mander et al. 2012). In contrast, a previous study found much higher and more variable CH_4 emissions (median 5.73 g C m^{-2} year^{-1} and range -0.01 to 113.21 g C m^{-2} year^{-1}, respectively) from the Lavassaare natural peatland area during two vegetation periods. These differences between disturbed and natural sites in CH_4 fluxes can be attributed to different substrate quality as well as lowered groundwater level and concordant changes in redox conditions in the peat column that inhibit the activity of methanogens and enhance CH_4 oxidising methanotrophs (Le Mer & Roger 2001, Andersen et al. 2013).

The results from bioenergy crop cultivation study (Paper I) show that reed canary grass cultivation mitigated CH_4 emission. Similar conclusion was also made by Hyvönen et al. (2009). However, methanogenic archaeal abundance remained approximately the same or even increased in different layers of residual peat under cultivated sites over time. This fact may indicate that methanogens were not very active in cultivated peat or methanotrophic bacteria, supported by plant exudates, consumed most of the CH_4. Furthermore, the increased bacterial abundance in the top layer of cultivated peat probably caused a consumption of CH_4 which was produced at lower layers of anaerobic sites and led to the decrease in CH_4 emission from these soils (Nazaries et al. 2013). As indicated by the positive relationships between *mcrA* gene abundance and gas emission in uncultivated peat, the two lower layers contributed the most to the CH_4 emission while in cultivated peat such types of relationships were not revealed. Juottonen et al. (2012) showed a similar relationship in the anaerobic (7.5–15 cm) layer of restored forestry drained boreal peatlands.

Fertilisation had little influence on CH_4 emission, and a few relationships between the emission and gene parameters in abandoned peat extraction area were found (Paper I). Nevertheless, several studies have shown that inorganic fertilisers inhibit methanotrophs while stimulating methanogens in various environments such as rice paddies, grasslands and forests (reviewed by Nazaries et al. 2013). Moreover, the addition of mineral nitrogen may alleviate nitrogen limitation for methanogens (Bodelier 2011, Singh et al. 2012), but the effect can vary in different ecosystems. Kim et al. (2015) showed that combined nitrogen and phosphorus addition decreased CH_4 production in the top layer (0–5 cm) of drainage ditch sediment due to increased substrate competition with denitrifiers. A large part of the uncertainty in the effect of nitrogen on CH_4 emission can

probably be explained by the varying plant responses which depend on plant species, environmental conditions, and on the type and amount of fertiliser as was shown in agricultural wetlands (Bodelier & Steenbergh 2014).

In addition, the results showed an increase in the bacterial community abundance in response to the reed canary grass cultivation, while the addition of nutrients did not have an effect on bacterial abundance (Paper I). Tavi et al. (2010) also showed a significant increase in microbial biomass and activity in the surface layer (0–15 cm) of reed canary grass-cultivated cutover peatland compared to the bare peat site. The archaeal proportion in the prokaryotic community was higher in uncultivated soils; the archaeal abundance was similar in cultivated and uncultivated peat. As a result of mineral fertiliser application, an increase was observed in archaeal abundance, especially in the deepest layer (40–60 cm) of cultivated peat.

The methanogen abundances were not different in respective layers of cultivated and uncultivated soils (Paper I). Niu et al. (2015) found also that cultivation of different typical water-tolerant plant species did not have a discernible effect on the abundance of methanogens in constructed wetlands. The effect of fertilisation was revealed in the 40–60 cm layer of fertilised cultivated peat where these organisms were more abundant, compared to the same layer of the unfertilised cultivated peat. This result may be explained by the fact that fertilisation boosted plant growth and carbon from plant roots and anoxic conditions probably provided favourable conditions for methanogens in this layer. The results of this study indicate that the addition of mineral fertiliser affected microbial relationships in uncultivated plots, while several relation-ships emerged between gene parameters after fertilisation.

4.3 Application of peatlands for wastewater treatment

Over the last century, an increasingly industrialised global economy has led to dramatically elevated releases of anthropogenic chemicals through wastewater into environment and resulted in contamination of many areas on Earth (Paper IV). In addition to other chemicals, the removal of excessive nitrogen and phosphorus from municipal and industrial wastewater as well as from polluted surface and subsurface waters has gained considerable attention to protect aquatic ecosystems primarily from pH changes, eutrophication and avoid adverse effects on human health and the economy (Camargo & Alonso 2006, Ramasahayam et al. 2014). It is vital to reduce the concentration of pollutants in the effluents of treatment systems to a level that does not cause problems in the environment. Treatment wetlands are considered as effective and low-cost solutions to protect aquatic ecosystems from pollution. These systems are used to decrease concentrations of pollutants in waters by implementing a combi-nation of physical, chemical and biological processes (Paper IV). In constructed treatment wetlands, most common filter materials are sand, gravel, limestone and light-weight clay aggregates, but peat can be an effective material for some

purification processes (Vohla et al. 2011). In contrast to the constructed treatment wetlands, there is much less information about natural treatment wetlands and their application for wastewater treatment.

Wetland treatment systems constructed on pristine peatlands have been successfully used to purify point source pollutants. Peatlands have a good potential to improve water treatment at a fairly low cost in the boreal region, where these ecosystems are abundant. For example, about 200 treatment peatlands for purifying peat extraction runoff were in use in Finland at the turn of the 21st century (Ronkanen & Kløve 2009). In these treatment peatlands, the phosphorus adsorption capacity has remained at a high level after almost 20 years of wetland use. Precipitation, sorption and peat accretion are the main processes affecting phosphorus retention in this kind of treatment wetlands (Karjalainen et al. 2016). Pure peat has a low capacity of phosphorus adsorption due to the negative charge of humic substances; however, it is well known that humic substances increase phosphorus adsorption via metal cations. Additionally, the retention efficiency may decrease when the loading of pollutants is very high (Ronkanen & Kløve 2009).

A natural wetland complex is capable of ameliorating pyrite mine waters rich in sulphur and metals, but cannot neutralise the acidity (Räisänen et al. 2001). Palmer et al. (2015) showed that the pre-treated process effluent and drainage waters of a gold mine containing high concentrations of arsenic, antimony and nickel can be purified successfully in treatment peatlands but the treatment efficiency may vary temporarily. Most of the contaminants were retained close to the wastewater distribution ditch and there was a near-linear increase in pollutant concentrations in the peatland over time. Due to the accumulation of contaminants, there is a limited lifetime for the treatment peatlands at the mine and increased risk of contaminant leaching after mine closure.

Peatland buffer areas are important in reducing sediment and nutrient loading from discharge waters of forested catchments; however, the use of natural mires as buffer areas may induce unwanted changes in the composition of plant species (Silvan et al. 2004, Saari et al. 2010, Hynninen et al. 2011, Nieminen et al. 2014). Vegetation acts also as a sink for pollutants, thus vegetation type and density may affect the retention efficiency of the compounds (Paper IV). Buffer areas bigger than 1% of the catchment area have been proven to be effective in water purification, while otherwise the short water residence time may significantly decrease their retention capacity (Nieminen et al. 2014).

The use of peatlands as treatment systems raises another concern regarding emissions of greenhouse gases such as N_2O and CH_4. The hydrological conditions in treatment peatland areas are different from natural and drained peat soils, namely, water level is generally above soil level and surface waters are in constant movement across the treatment peatland area. As nutrient inputs to treatment peatland areas can also be larger than those in other types of peat soils, the N_2O and CH_4 emissions measured in natural or drained peatlands cannot be applied to these areas. Natural treatment wetlands have not yet been extensively studied in perspective of microbial community or greenhouse gases.

5. CONCLUSIONS

Natural peatlands are unique ecosystems that provide functions like carbon and nitrogen stock and balanced hydrology. Among other functions, peatlands are used by humans worldwide for agriculture, forestry, fuel production and pollution control purposes. Most of the management practices create alterations in hydrological regimes, which initiate a cascade of changes in peat, and affect the form and function of peatlands. An investigation of managed peatlands is necessary to reveal and assess the economical, ecological and environmental effects, especially in less studied regions such as tropics. The results presented in this dissertation allow to make the following conclusions:

Natural northern peatlands are important sources of CH_4 emissions to the atmosphere, although emissions between the sites were highly variable. Peatland type, pH, water table depth and air temperature are important factors in determining CH_4 flux in natural sites. The drainage practices in northern peatlands clearly reduce the CH_4 flux, on average, by 84%. Additionally, drainage influences CH_4 emissions from fens more than from bogs.

Greenhouse gas emissions were also affected by drainage in tropical peatland sites. The average CH_4 flux from the natural site was higher than that for the drained and the average N_2O flux from the natural site was lower than that for the drained. The potential for N_2 emission was greater in the top soil layer at the natural site compared to the drained site. Drainage changed the balance between bacterial and archaeal groups in the soil in favour of plant biomass-decomposing microbial communities, especially towards *Actinobacteria*, *Firmicutes*, *Crenarchaeota* and euryarchaeal class *Thermoplasmata*. In addition, the drainage of a tropical peatland led to other structural changes in the soil bacterial and archaeal communities, also reflected in alterations in *nirK*, *nirS*, *nosZ*, *nifH* and archaeal *amoA* gene-possessing microbial communities. The drainage reduced the denitrification and N_2-fixing potential in the tropical peatland. In undisturbed peatland soil, the N_2O emission was primarily related to *nirS*-type denitrifiers and DNRA, while the conversion of N_2O to N_2 was controlled by *nosZ* clade I genes. The drainage induced changes in the soil denitrifying microbial community, and the main reducers of N_2O were microbes harbouring *nosZ* clade II genes.

Restoration of drained northern peatlands by rewetting or simultaneous vegetation and rewetting increases CH_4 emissions, on average, by 46% compared to the pre-restoration CH_4 fluxes. Nevertheless, reed canary grass cultivation in residual peat of an abandoned peat extraction area reduced CH_4 emission, although methanogen abundance remained approximately the same or even increased in different layers of residual peat under cultivated sites over time. In general, the methanogen abundance was low in the archaeal community, but the highest CH_4 production potential was revealed at the bottom of the peat column at abandoned peat extraction area. In uncultivated peat, the two deeper layers influenced mainly the CH_4 emission, while in cultivated peat, the

more pronounced effect of methanotrophic bacteria on CH_4 emission can be assumed from the obtained results. Additionally, the physicochemical status of peat was slightly changed and bacterial abundance increased in response to the reed canary grass cultivation.

Peatlands have a good potential to improve water treatment at a fairly low cost in peatland-rich regions in order to protect aquatic ecosystems from pollution. Natural treatment wetlands have been used to treat many types of contaminants including excessive nitrogen, phosphorus, suspended solids, sulphur and metals. Besides peat itself, vegetation acts also as a sink for pollutants, thus vegetation type and density affect the compounds retention efficiency.

Based on the results of this study, it can be concluded that the exploitation of peatlands change considerably the soil microbiome and greenhouse gas emissions, although some management practices alleviate environmental damage.

6. REFERENCES

Aerts R, Verhoeven JTA, Whigham DF (1999) Plant-mediated controls on nutrient cycling in temperate fens and bogs. *Ecology* 80: 2170–2181.

Albert T, Karp K, Starast M, Moor U, Paal T (2011) Effect of Fertilization on the Lowbush Blueberry Productivity and Fruit Composition in Peat Soil. *Journal of Plant Nutrition* 34: 1489–1496.

Alexander PD, Bragg NC, Meade R, Padelopoulos G, Watts O (2008). Peat in horticulture and conservation: the UK response to a changing world. *Mires and Peat* 3: 1–10.

Andersen R, Francez AJ, Rochefort L (2006) The physicochemical and microbiological status of a restored bog in Québec: Identification of relevant criteria to monitor success. *Soil Biology & Biochemistry* 38: 1375–1387.

Andersen R, Chapman SJ, Artz RRE (2013) Microbial communities in natural and disturbed peatlands: A review. *Soil Biology & Biochemistry* 57: 979–994.

Aronson EL, Allison SD, Helliker BR (2013) Environmental impacts on the diversity of methane-cycling microbes and their resultant function. *Frontiers in microbiology* 4: 1–15.

Baird AJ, Belyea L, Morris PJ (2009) Upscaling peatland-atmosphere fluxes of carbon gases: Small-scale heterogeneity in process rates and the pitfalls of 'bucket-and-slab' models. In: *Northern Peatlands and carbon cycling* (Eds. Baird AJ, Belyea LR, Comas X, Reeve A, Slater L), pp. 37–53, USA, American Geophysical Union Monograph.

Basiliko N, Knowles R, Moore TR (2004) Roles of moss species and habitats in methane consumption potential in a northern peatland. *Wetlands* 24: 178–185.

Basiliko N, Blodau C, Roehm C, Bengtson P, Moore TR (2007) Regulation of Decomposition and Methane Dynamics across Natural, Commercially Mined, and Restored Northern Peatlands. *Ecosystems* 10: 1148–1165.

Beer A-M, Junginger HE, Lukanov J, Sagorchev P (2003) Evaluation of the permeation of peat substances through human skin in vitro. *International Journal of Pharmaceutics* 253: 169–175.

Beetz S, Liebersbach H, Glatzel S, Jurasinski G, Buczko U, Höper H (2013) Effects of land use intensity on the full greenhouse gas balance in an Atlantic peat bog. *Biogeosciences* 10: 1067–1082.

Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology* 68(1): 1–13.

Berglund Ö, Berglund K (2011) Influence of water table level and soil properties on emissions of greenhouse gases from cultivated peat soil. *Soil Biology & Biochemistry* 43: 923–931.

Bergmann GT, Bates ST, Eilers KG, Lauber CL, Caporaso JG, Walters WA, Knight R, Fierer N (2011) The under-recognized dominance of *Verrucomicrobia* in soil bacterial communities. *Soil Biology and Biochemistry* 43(7): 1450–1455.

Bodelier PLE (2011) Interactions between nitrogenous fertilizers and methane cycling in wetland and upland soils. *Current Opinion in Environmental Sustainability* 3: 379–388.

Bodelier PLE, Steenbergh AK (2014) Interactions between methane and the nitrogen cycle in light of climate change. *Current Opinion in Environmental Sustainability* 9–10: 26–36.

Bridgham SD, Cadillo-Quiroz H, Keller JK, Zhuang Q (2013) Methane emissions from wetlands: biogeochemical, microbial, and modeling perspectives from local to global scales. *Global Change Biology* 19(5): 1325–1346.

Brown PA, Gill SA, Allen SJ (2000) Metal removal from wastewater using peat. *Water Research* 34(16): 3907–3916.

Butterbach-Bahl K, Gundersen P, Ambus P, Augustin J, Beier C, Boeckx P, Dannenmann M, Sanchez Gimeno B, Ibrom A, Kiese R, Kitzler B (2011) Nitrogen processes in terrestrial ecosystems. In: *The European nitrogen assessment: sources, effects and policy perspectives* (Eds. Sutton MA, Howard CM, Erisman JW, Billen G, Bleeker A, Grennfelt P, van Grinsven H, Grizzetti B), pp. 99–125, UK, Cambridge University Press.

Cabello P, Roldán MD, Moreno-Vivián C (2004) Nitrate reduction and the nitrogen cycle in archaea. *Microbiology* 150: 3527–3546.

Caisse G, Boudreau S, Munson AD, Rochefort L (2008) Fertiliser addition is important for tree growth on cut-over peatlands in eastern Canada. *Mires and Peat* 3: 1–15.

Camargo JA, Alonso Á (2006) Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. *Environment international* 32(6): 831–849.

Chen P, Li J, Li QX, Wang Y, Li S, Ren T, Wang L (2012) Simultaneous heterotrophic nitrification and aerobic denitrification by bacterium *Rhodococcus sp. CPZ24*. *Bioresource Technology* 116: 266–270.

Costa AS, Romćo LPC, Araśjo BR, Lucas SCO, Maciel STA, Wisniewski Jr. A, Alexandre MR (2012) Environmental strategies to remove volatile aromatic fractions (BTEX) from petroleum industry wastewater using biomass. *Bioresource Technology* 105: 31–39.

Cubizolle H, Mayindza Mouandza M, Muller F (2013) Mires and Histosols in French Guiana (South America): new data relating to location and area. *Mires and Peat* 12: 1–10.

Daims H, Lebedeva EV, Pjevac P, Han P, Herbold C, Albertsen M, Jehmlich N, Palatinszky M, Vierheilig J, Bulaev A, Kirkegaard RH (2015) Complete nitrification by *Nitrospira* bacteria. *Nature* 528(7583): 504–509.

Dargie GC, Lewis SL, Lawson IT, Mitchard ET, Page SE, Bocko YE, Ifo SA (2017) Age, extent and carbon storage of the central Congo Basin peatland complex. *Nature* 542(7639): 86–90.

Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440(7081): 165–173.

Decleyre H, Heylen K, Tytgat B, Willems A (2016) Highly diverse *nirK* genes comprise two major clades that harbour ammonium-producing denitrifiers. *BMC genomics* 17(1): 155.

Dedysh SN (2011) Cultivating uncultured bacteria from northern wetlands: knowledge gained and remaining gaps. *Frontiers in microbiology* 2: 184.

Dignac MF, Derrien D, Barré P, Barot S, Cécillon L, Chenu C, Chevallier T, Freschet GT, Garnier P, Guenet B, Hedde M, Klumpp K, Lashermes G, Maron P-A, Nunan N, Roumet C, Basile-Doelsch I (2017) Increasing soil carbon storage: mechanisms, effects of agricultural practices and proxies. A review. *Agronomy for Sustainable Development* 37(2): 14.

Ding WX, Cai ZC (2007) Methane emission from natural wetlands in China: Summary of years 1995–2004 studies. *Pedosphere* 17: 475–485.

Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Mangenot S, Kuypers MM, Schreiber F, Dutilh BE, Zedelius J, de Beer D, Gloerich J, Wessels HJ, van Alen T, Luesken F, Wu ML, van de Pas-Schoonen KT, Op den Camp HJ, Janssen-Megens EM, Francoijs KJ, Stunnenberg H, Weissenbach J, Jetten MS, Strous M (2010). Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature* 464(7288): 543–548.

Fonder N, Headley T (2013) The taxonomy of treatment wetlands: A proposed classification and nomenclature system. *Ecological Engineering* 51: 203–211.

Frenzel P, Karofeld E (2000) CH_4 emission from a hollow-ridge complex in a raised bog: The role of CH_4 production and oxidation. *Biogeochemistry* 51: 91–112.

Fuerst JA, Sagulenko E (2011) Beyond the bacterium: planctomycetes challenge our concepts of microbial structure and function. *Nature Reviews Microbiology* 9(6): 403–413.

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of the Nitrogen Cycle: Recent Trends, Questions and Potential Solutions. *Science* 320: 889–892.

Ghica A, Dragomir C, Samfira I (2012) *Phalaris Arundinacea* a further energetic species. *Research Journal of Agricultural Science* 44(4): 46–51.

Graf MD, Rochefort L, Poulin M (2008) Spontaneous revegetation of cutaway peatlands of North America. *Wetlands* 28: 28–39.

Graf DRH, Jones CM, Hallin S (2014) Intergenomic comparisons highlight modularity of the denitrification pathway and underpin the importance of community structure for N_2O emissions. *PLoS ONE* 9: e114118.

Granberg G, Mikkelä C, Sundh I, Svensson BH, Nilsson M (1997) Source of spatial variation in methane emission from mires in northern Sweden: A mechanistic approach in statistical modeling. *Global Biogeochemical Cycles* 11: 135–150.

Greenup AL, Bradford MA, McNamara NP, Ineson P, Lee JA (2000) The role of *Eriophorum vaginatum* in CH_4 flux from an ombrotrophic peatland. *Plant and Soil* 227: 265–272.

Gruber N, Galloway JN (2008) An Earth-system perspective of the global nitrogen cycle. *Nature* 451(7176): 293–296.

Gumbricht T, Roman-Cuesta RM, Verchot L, Herold M, Wittmann F, Householder E, Herold N, Murdiyarso D (2017) An expert system model for mapping tropical wetlands and peatlands reveals South America as the largest contributor. *Global Change Biology* 00: 1–19.

Han MV, Zmasek CM (2009) phyloXML: XML for evolutionary biology and comparative genomics. *BMC Bioinformatics* 10: 356.

Harhangi HR, Le Roy M, van Alen T, Hu BL, Groen J, Kartal B, Tringe SG, Quan ZX, Jetten MS, Op den Camp HJ (2012) Hydrazine synthase, a unique phylomarker with which to study the presence and biodiversity of anammox bacteria. *Applied and environmental microbiology* 78(3): 752–758.

Hatano R, Toma Y, Hamada Y, Arai H, Susilawati HL, Inubushi K (2016) Methane and Nitrous Oxide Emissions from Tropical Peat Soil. In: *Tropical Peatland Ecosystems* (Eds. Osaki M, Tsuji N), pp. 339–351, Japan, Springer.

Hatzenpichler R (2012) Diversity, physiology, and niche differentiation of ammonia-oxidizing archaea. *Applied and Environmental Microbiology* 78(21): 7501–7510.

Heinsoo K, Hein K, Melts I, Holm B, Ivask M (2011) Reed canary grass yield and fuel quality in Estonian farmers' fields. *Biomass and Bioenergy* 35: 617–625.

Herlemann DP, Lundin D, Labrenz M, Jürgens K, Zheng Z, Aspeborg H, Andersson AF (2013) Metagenomic *de novo* assembly of an aquatic representative of the verrucomicrobial class *Spartobacteria*. *mBio* 4(3): e00569–12.

Higgins T, Colleran E (2006) Trophic Status of Experimental Cutaway Peatland Lakes in Ireland and Implications for Future Lake Creation. *Journal of Environmental Science and Health, Part A* 41: 849–863.

Hiraishi T, Krug T, Tanabe K, Srivastava N, Baasansuren J, Fukuda M, Troxler TG (2014) 2013 supplement to the 2006 IPCC guidelines for national greenhouse gas inventories: Wetlands, Switzerland, IPCC.

Holmes AJ, Costello A, Lidstrom ME, Murrell JC (1995) Evidence that particulate methane monooxygenase and ammonia monooxygenase may be evolutionary related. *FEMS Microbiology Letters* 132: 203–208.

Hu BL, Rush D, van der Biezen E, Zheng P, van Mullekom M, Schouten S, Damsté JSS, Smolders AJ, Jetten MS, Kartal B (2011a) New anaerobic, ammonium-oxidizing community enriched from peat soil. *Applied and environmental micro-biology* 77(3): 966–971.

Hu BL, Shen LD, Xu XY, Zheng P (2011b) Anaerobic ammonium oxidation (anammox) in different natural ecosystems. *Biochemical Society Transactions* 39: 1811–1816.

Huotari N, Tillman-Sutela E, Kauppi A, Kubin E (2007) Fertilization ensures rapid formation of ground vegetation on cut-away peatlands. *Canadian Journal of Forest Research* 37: 874–883.

Hutchinson GL, Livingston GP (1993) Use of Chamber Systems to Measure Trace Gas Fluxes. In: *Agricultural Ecosystem Effects on Trace Gases and Global Climate Change* (Eds. Rolston DE, Duxbury JM, Harper LA, Mosier AR), pp. 63–78, USA, ASA Spec. Publ. 55. ASA, CSSA, and SSSA.

Hutsch BW (1998) Tillage and land use effects on methane oxidation rates and their vertical profiles in soil. *Biology and Fertility of Soils* 27(3): 284–292.

Huttunen JT, Nykänen H, Turunen J, Martikainen PJ (2003) Methane emissions from natural peatlands in the northern boreal zone in Finland. *Fennoscandia Atmospheric Environment* 37: 147–151.

Hynninen A, Hamberg L, Nousiainen H, Korpela L, Nieminen M (2011) Vegetation composition dynamics in peatlands used as buffer areas in forested catchments in southern and central Finland. *Plant ecology* 212(11): 1803.

Hyvönen NP, Huttunen JT, Shurpali NJ, Tavi NM, Repo ME, Martikainen PJ (2009) Fluxes of nitrous oxide and methane on an abandoned peat extraction site: Effect of reed canary grass cultivation. *Bioresource Technology* 100: 4723–4730.

IPCC (2013) Climate change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (Eds. Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM), UK, USA, Cambridge University Press.

Järveoja J, Laht J, Maddison M, Soosaar K, Ostonen I, Mander Ü (2012) Mitigation of greenhouse gas emissions from an abandoned Baltic peat extraction area by growing reed canary grass: life-cycle assessment. *Regional Environmental Change* 13(4): 781–795.

Jensen MM, Kuypers MMM, Lavik G, Thamdrup B (2008) Rates and regulation of anaerobic ammonium oxidation and denitrification in the Black Sea. *Limnology and Oceanography* 53: 23–36.

Jetten MSM, Op den Camp HJM, Kuenen JG, Strous M (2010) Description of the order *Brocadiales*. In: *Bergey's Manual of Systematic Bacteriology: The Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes* (Eds. Krieg NR, Ludwig W, Whitman WB, Hedlund BP, Paster BJ, Staley JT, Ward N, Brown D, Parte A), pp. 596–603, USA, Springer.

Jones CM, Stres B, Rosenquist M, Hallin S (2008) Phylogenetic analysis of nitrite, nitric oxide, and nitrous oxide respiratory enzymes reveal a complex evolutionary history for denitrification. *Molecular Biology and Evolution* 25(9): 1955–1966.

Jones CM, Graf DRH, Bru D, Philippot L, Hallin S (2013) The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. *The ISME Journal* 7: 417–426.

Joosten H, Clarke D (2002) Wise use of mires and peatlands: background and principles including a framework for decision-making. Finland, International Mire Conservation Group and Internatonal Peat Society.

Juottonen H, Galand PE, Tuittila ES, Laine J, Fritze H, Yrjälä K (2005) Methanogen communities and bacteria along an ecohydrological gradient in a northern raised bog complex. *Environmental Microbiology* 7: 1547–1557.

Juottonen H, Galand PE, Yrjälä K (2006) Detection of methanogenic Archaea in peat: comparison of PCR primers targeting the *mcrA* gene. *Research in Microbiology* 157: 914–921.

Juottonen H, Hynninen A, Nieminen M, Tuomivirta TT, Tuittila ES, Nousiainen H, Kell DK, Yrjälä K, Tervahauta A, Fritze H (2012) Methane-cycling microbial communities and methane emission in natural and restored peatlands. *Applied and environmental microbiology* 78: 6386–6389.

Kadlec RH, Wallace S (2009) Treatment wetlands. USA, CRC press.

Karjalainen SM, Heikkinen K, Ihme R, Kløve B (2016) Long-term purification efficiency of a wetland constructed to treat runoff from peat extraction. *Journal of Environmental Science and Health, Part A* 51(5): 393–402.

Kasak K, Mander Ü, Truu J, Truu M, Järveoja J, Maddison M, Teemusk A (2015) Alternative filter material removes phosphorus and mitigates greenhouse gas emission in horizontal subsurface flow filters for wastewater treatment. *Ecological Engineering* 77: 242–249.

Kasak K, Mõtlep R, Truu M, Truu J, Kõiv-Vainik M, Espenberg M, Paiste P, Kirsimäe K, Mander Ü (2016) Hydrated Oil Shale Ash Mitigates Greenhouse Gas Emissions from Horizontal Subsurface Flow Filters for Wastewater Treatment. *Water, Air, & Soil Pollution* 227(9): 320.

Kersters K, De Vos P, Gillis M, Swings J, Vandamme P, Stackebrandt E (2006) Introduction to the *Proteobacteria*. In: *The Prokaryotes*, 3rd edition (Eds. Dwarkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E), pp. 3–37, USA, Springer.

Kielak AM, Barreto CC, Kowalchuk GA, van Veen JA, Kuramae EE (2016) The ecology of *Acidobacteria*: moving beyond genes and genomes. *Frontiers in Microbiology* 7: 744.

Kim SY, Veraart AJ, Meima-Franke M, Bodelier PLE (2015) Combined effects of carbon, nitrogen and phosphorus on CH4 production and denitrification in wetland sediments. *Geoderma* 259: 354–361.

Klavins M, Kokorite I, Springe G, Skuja A, Parele E, Rodinov V, Druvietis I, Strake S, Urtans A (2010) Water quality in cutaway peatland lakes in Seda mire, Latvia. *Ecohydrology & Hydrobiology* 10: 61–70.

Kõiv M, Vohla C, Mõtlep R, Liira M, Kirsimäe K, Mander Ü (2009) The performance of peat-filled subsurface flow filters treating landfill leachate and municipal wastewater. *Ecological Engineering* 35: 204–212.

Komulainen VM, Nykänen H, Martikainen PJ, Laine J (1998) Short-term effect of restoration on vegetation change and methane emissions from peatlands drained for forestry in southern Finland. *Canadian Journal of Forest Research* 28(3): 402–411.

Könneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* 437: 543–546.

Könneke M, Schubert DM, Brown PC, Hügler M, Standfest S, Schwander T, von Borzyskowski LS, Erb TJ, Stahl DA, Berg IA (2014) Ammonia-oxidizing archaea use the most energy-efficient aerobic pathway for CO2 fixation. *Proceedings of the National Academy of Sciences* 111(22): 8239–8244.

Koskinen M, Tahvanainen T, Sarkkola S, Menberu MW, Laurén A, Sallantaus T, Marttila H, Ronkanen AK, Parviainen M, Tolvanen A, Koivusalo H (2017) Restoration of nutrient-rich forestry-drained peatlands poses a risk for high exports of dissolved organic carbon, nitrogen, and phosphorus. *Science of The Total Environment* 586: 858–869.

Lam P, Kuypers MM (2011) Microbial nitrogen cycling processes in oxygen minimum zones. *Annual review of marine science* 3: 317–345.

Le Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: A review. *European Journal of Soil Biology* 37: 25–50.

Lewin GR, Carlos C, Chevrette MG, Horn HA, McDonald BR, Stankey RJ, Fox BG, Currie CR (2016) Evolution and Ecology of *Actinobacteria* and Their Bioenergy Applications. *Annual Review of Microbiology* 70: 235–254.

Ligi T, Truu M, Oopkaup K, Nõlvak H, Mander Ü, Mitsch WJ, Truu J (2014). The genetic potential of N_2 emission via denitrification and ANAMMOX from the soils and sediments of a created riverine treatment wetland complex. *Ecological Engineering* 80: 181–190.

Ligi T (2015) Bacterial community structure and its genetic potential for nitrogen removal in the soils and sediments of a created riverine wetland complex. *Dissertationes Technologiae Circumiectorum Universitatis Tartuensis* (Doctoral dissertation).

Limpens J, Berendse F, Blodau C, Canadell JG, Freeman C, Holden J, Roulet N, Rydin H, Schaepman-Strub G (2008) Peatlands and the carbon cycle: from local processes to global implications–a synthesis. *Biogeosciences* 5(5): 1475–1491.

Lin X, Tfaily MM, Green SJ, Steinweg JM, Chanton P, Imvittaya A, Chanton JP, Cooper W, Schadt C, Kostka JE (2014) Microbial metabolic potential for carbon degradation and nutrient (nitrogen and phosphorus) acquisition in an ombrotrophic peatland. *Applied and environmental microbiology* 80(11): 3531–3540.

Lüke C, Speth DR, Kox MAR, Villanueva L, Jetten MSM (2016) Metagenomic analysis of nitrogen and methane cycling in the Arabian Sea oxygen minimum zone. *PeerJ* 4: e1924.

Mahmood MDS, Strack M (2011) Methane dynamics of recolonized cutover minerotrophic peatland: Implications for restoration. *Ecological Engineering* 37: 1859–1868.

Maljanen M, Sigurdsson BD, Gudmundsson J, Oskarsson H, Huttunen JT, Martikainen PJ (2010) Greenhouse gas balances of managed peatlands in the Nordic countries – present knowledge and gaps. *Biogeosciences* 7: 2711–2738.

Mander Ü, Järveoja J, Maddison M, Soosaar K, Aavola R, Ostonen I, Salm (JO 2012) Reed canary grass cultivation mitigates greenhouse gas emissions from abandoned peat extraction areas. *GCB Bioenergy* 4: 462–474.

Mandic-Mulec I, Ausec L, Danevcic T, Levicnik-Hoefferle S, Jerman V, Kraigher B (2014) Microbial community structure and function in peat soil. *Food Technology and Biotechnology* 52(2): 180–187.

Mania D, Heylen K, Spanning RJ, Frostegård Å (2014) The nitrate-ammonifying and *nosZ*-carrying bacterium *Bacillus vireti* is a potent source and sink for nitric and nitrous oxide under high nitrate conditions. *Environmental microbiology* 16(10): 3196–3210.

Martikainen PJ, Nykanen H, Crill P, Silvola J (1993) Effect of a lowered water-table on nitrous-oxide fluxes from northern peatlands. *Nature* 366: 51–53.

McDonald IR, Bodrossy L, Chen Y, Murrell JC (2008) Molecular ecology techniques for the study of aerobic methanotrophs. *Applied and Environmental Microbiology* 74(5): 1305–1315.

Menzel P, Ng KL, Krogh A (2016) Fast and sensitive taxonomic classification for metagenomics with Kaiju. *Nature Communications* 7: 11257.

Minkkinen K, Byrne KA, Trettin C (2008) Climate impacts of peatland forestry. In: *Peatlands and climate change* (Ed. Strack M), pp. 98–122, Finland, International Peat Society.

Mitsch WJ, Gosselink JG (2015) Wetlands. 5th edition. USA, Wiley.

Moore TR, Dalva M (2006) The influence of temperature and water table position on carbon dioxide and methane emissions from laboratory columns of peatland soils. *Journal of Soil Science* 44: 651–664.

Mulder A, Vandegraaf AA, Robertson LA, Kuenen JG (1995) Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. *FEMS microbiology ecology* 16: 177–183.

Nazaries L, Murrell JC, Millard P, Baggs L, Singh BK (2013) Methane, microbes and models: Fundamental understanding of the soil methane cycle for future predictions. *Environmental Microbiology* 15: 2395–2417.

Nelson MB, Martiny AC, Martiny JB (2016) Global biogeography of microbial nitrogen-cycling traits in soil. *Proceedings of the National Academy of Sciences* 113(29): 8033–8040.

Nieminen M, Kaila A, Koskinen M, Sarkkola S, Fritze H, Tuittila E-S, Nousiainen H, Koivusalo H, Laurén A, Ilvesniemi H, Vasander H, Sallantaus T (2014) Natural and restored wetland buffers in reducing sediment and nutrient export from forested catchments: Finnish experiences. In: *The role of natural and constructed wetlands in nutrient cycling and retention on the landscape* (Ed. Vymazal J), Switzerland, Springer.

Niu C, He Z, Ge Y, Chang J, Lu Z (2015) Effect of plant species richness on methane fluxes and associated microbial processes in wetland microcosms. *Ecological engineering* 84: 250–259.

Nykänen H, Alm J, Silvola J, Tolonen K, Martikainen PJ (1998) Methane fluxes on boreal peatlands of different fertility and the effect of long-term experimental lowering of the water table on flux rates. *Global Biogeochemical Cycles* 12: 53–69.

Offre P, Spang A, Schleper C (2013) Archaea in Biogeochemical Cycles. *Annual Review of Microbiology* 67: 437–457.

Oleszczuk R, Regina K, Szajdak H, Höper H, Maryganova V (2008) Impacts of agricultural utilization of peat soils on the greenhouse gas balance. In: *Peatlands and climate change* (Ed. Strack M), pp. 70–97, Finland, International Peat Society

Orru M, Übner M, Orru H (2011) Chemical properties of peat in three peatlands with balneological potential in Estonia. *Estonian Journal of Earth Sciences* 60: 43–49.

Oton EV, Quince C, Nicol GW, Prosser JI, Gubry-Rangin C (2016) Phylogenetic congruence and ecological coherence in terrestrial *Thaumarchaeota*. *The ISME journal* 10(1): 85–96.

Paal J (2011) Jääksood, nende kasutamine ja korrastamine [In Estonian]. Estonia, Eesti Turbaliit.

Paal J, Leibak E (2011) Estonian mires: inventory of habitats. Publication of the Project "Estonian Mires Inventory completion for maintaining biodiversity". Estonia, Regio.

Page SE, Rieley JO, Banks CJ (2011) Global and regional importance of the tropical peatland carbon pool. *Global Change Biology* 17(2): 798–818.

Pajares S, Bohannan BJM (2016) Ecology of Nitrogen Fixing, Nitrifying, and Denitrifying Microorganisms in Tropical Forest Soils. *Frontiers in Microbiology* 7: 1045.

Palmer K, Ronkanen A-K, Kløve B (2015) Efficient removal of arsenic, antimony and nickel from mine wastewaters in Northern treatment peatlands and potential risks in their long-term use. *Ecological Engineering* 75: 350–364.

Pjevac P, Schauberger C, Poghosyan L, Herbold CW, van Kessel MA, Daebeler A, Steinberger M, Jetten MS, Lücker S, Wagner M, Daims H (2016) *AmoA*-targeted polymerase chain reaction primers for the specific detection and quantification of comammox *Nitrospira* in the environment. *bioRxiv*: 096891.

Raghoebarsing AA, Smolders AJP, Schmid MC, Rijpstra WIC, Wolters-Arts M, Derksen J, Jetten MSM, Schouten S, Sinninghe Damste JS, Lamers LPM, Roelofs JGM, Op den Camp HJM, Strous M (2005) Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. *Nature* 436: 1153–1156.

Raghoebarsing AA, Pol A, van de Pas-Schoonen KT, Smolders AJ, Ettwig KF, Rijpstra WI, Schouten S, Sinninghe Damsté JS, Op den Camp HJ, Jetten MS, Strous M (2006) A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature* 440(7086): 918–921.

Räisänen ML, Lestinen P, Kuivasaari T (2001) The retention of metals and sulphur in a natural wetland – preliminary results from the old Otravaara pyrite mine, eastern Finland. In: *Proceeding of International Conference on Mining and the Environment, Skellefteå, Sweden vol. 2*, pp. 662–670, Sweden, The Swedish Mining Association.

Ramasahayam SK, Guzman L, Gunawan G, Viswanathan T (2014) A comprehensive review of phosphorus removal technologies and processes. *Journal of Macromolecular Science, Part A* 51(6): 538–545.

Reed SC, Cleveland CC, Townsend AR (2011) Functional ecology of free-living nitrogen fixation: a contemporary perspective. *Annual review of ecology, evolution, and systematics* 42: 489–512.

Ringqvist L, Holmgren A, Öborn I (2002) Poorly humified peat as an adsorbent for metals in wastewater. *Water Research* 36: 2394–2404.

Ronkanen AK, Kløve B (2009) Long-term phosphorus and nitrogen removal processes and preferential flow paths in Northern constructed peatlands. *Ecological Engineering* 35(5): 843–855.

Roulet NT, Ash R, Quinton W (1993) Methane flux from drained northern peatlands: Effect of a persistent water table lowering on flux. *Global Biogeochemical Cycles* 7: 749–769.

Roulet NT, Moore TR (1995) The effect of forestry drainage practices on the emission of methane from northern peatlands. *Candian Journal of Forest Research* 25: 491–499.

Rütting T, Boeckx P, Müller C, Klemedtsson L (2011) Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. *Biogeosciences* 8(7): 1779–1791.

Saari P, Saari V, Luotonen H, Alm J (2010) Vegetation change in peatland buffers as an indicator of active areas of run-on from forestry. *Annales Botanici Fennici* 47: 425–438.

Saarnio S, Alm J, Martikainen PJ, Silvola J (1998) Effects of raised CO_2 on potential CH_4 production and oxidation in, and CH_4 emission from, a boreal mire. *Journal of Ecology* 86: 261–268.

Samaritani E, Siegenthaler A, Yli-Petäys M, Buttler A, Christin P, Mitchell EAD (2011) Seasonal net ecosystem carbon exchange of a regenerating cutaway bog: How long does it take to restore the C-sequestration function? *Restoration Ecology* 19: 480–489.

Sanford RA, Wagner DD, Wu Q, Chee-Sanford JC, Thomas SH, Cruz-García C, Rodríguez G, Massol-Deyá A, Krishnani KK, Ritalahti KM, Nissen S (2012) Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils. *Proceedings of the National Academy of Sciences* 109(48): 19709–19714.

Schimel JP, Schaeffer SM (2012) Microbial control over carbon cycling in soil. *Frontiers in microbiology* 3: 1–11.

Schmidt MW, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kögel-Knabner I, Lehmann J, Manning DA, Nannipieri P (2011) Persistence of soil organic matter as an ecosystem property. *Nature* 478(7367): 49–56.

Schrier-Uijl AP, Veenendaal EM, Leffelaar PA, van Huissteden JC, Berendse F (2010) Methane emissions in two drained peat agro-ecosystems with high and low agricultural intensity. *Plant and Soil* 329: 509–520.

Shelef O, Gross A, Rachmilevitch S (2013) Role of plants in a constructed wetland: current and new perspectives. *Water* 5(2): 405–419.

Shoun H, Fushinobu S, Jiang L, Kim S, Wakagi T (2012) Fungal denitrification and nitric oxide reductase cytochrome P450nor. *Philosophical Transactions of the Royal Society B* 367: 1186–1194.

Silvan N, Vasander H, Laine J (2004) Vegetation is the main factor in nutrient retention in a constructed wetland buffer. *Plant and Soil* 258(1): 179–187.

Singh S, Singh JS, Kashyap AK (1999) Methane flux from irrigated rice fields in relation to crop growth and N-fertilization. *Soil Biology and Biochemistry* 31: 1219–1228.

Singh A, Singh RS, Upadhyay SN, Joshi CG, Tripathi AK, Dubey SK (2012) Community structure of methanogenic archaea and methane production associated with compost-treated tropical rice-field soil. *FEMS Microbiology Ecology* 82: 118–134.

Sjögersten S, Cheesman AW, Lopez O, Turner BL (2011) Biogeochemical processes along a nutrient gradient in a tropical ombrotrophic peatland. *Biogeochemistry* 104: 147–163.

Stahl DA, de la Torre JR (2012) Physiology and diversity of ammonia-oxidizing archaea. *Annual review of microbiology* 66: 83–101.

Stein LY, Klotz MG (2016) The nitrogen cycle. *Current Biology* 26(3): R94–R98.

Stone MM, Kan J, Plante AF (2015) Parent material and vegetation influence bacterial community structure and nitrogen functional genes along deep tropical soil profiles at the Luquillo Critical Zone Observatory. *Soil Biology & Biochemistry* 80: 273–282.

Strack M, Zuback YCA (2013) Annual carbon balance of a peatland 10 yr following restoration. *Biogeosciences* 10: 2885–2896.

Sundh I, Nilsson M, Mikkela C, Granberg G, Svensson H (2000) Fluxes of methane and carbon dioxide on peat-mining areas in Sweden. *AMBIO: A Journal of the Human Environment* 29: 499–503.

Suni S, Kosunen A-L, Hautala M, Pasila A, Romantschuk M (2004) Use of a by-product of peat excavation, cotton grass fibre, as a sorbent for oil-spills. *Marine Pollution Bulletin* 49: 916–921.

Tavi NM, Keinanen-Toivola MM, Koponen HT, Huttunen JT, Kekki TK, Biasi C, Martikainen PJ (2010) Impact of Phalaris arundinacea cultivation on microbial community of a cutover peatland. *Boreal environment research.*15: 437–445.

Templer PH, Silver WL, Pett-Ridge J, DeAngelis KM, Firestone MK (2008) Plant and microbial controls on nitrogen retention and loss in a humid tropical forest. *Ecology* 89: 3030–3040.

Tiedje JM (1988) Ecology of denitrification and dissimilatory nitrate reduction to ammonium. *Biology of anaerobic microorganisms* 717: 179–244.

Trivedi P, Delgado-Baquerizo M, Anderson IC, Singh BK (2016) Response of Soil Properties and Microbial Communities to Agriculture: Implications for Primary Productivity and Soil Health Indicators. *Frontiers in Plant Science* 7: 990.

Tuittila ES, Komulainen VM, Vasander H, Nykänen H, Martikainen PJ, Laine J (2000) Methane dynamics of a restored cut-away peatland. *Global Change Biology* 6: 569–581.

Urbanová Z, Bárta J (2016) Effects of long-term drainage on microbial community composition vary between peatland types. *Soil Biology & Biochemistry* 92: 16–26.

van Groenigen JW, Huygens D, Boeckx P, Kuyper TW, Lubbers IM, Rütting T, Groffman PM (2015) The soil N cycle: new insights and key challenges. *Soil* 1: 235–256.

van den Heuvel RN, van der Biezen E, Jetten MSM, Hefting MM, Kartal B (2010) Denitrification at pH 4 by a soil-derived *Rhodanobacter*-dominated community. *Environmental Microbiology* 12: 3264–3271.

van Kessel MA, Speth DR, Albertsen M, Nielsen PH, Op den Camp HJ, Kartal B, Jetten MS, Lücker S (2015) Complete nitrification by a single microorganism. *Nature* 528(7583): 555–559.

Virtanen K, Valpola S (2011) Energy potential of Finnish peatlands. *Geological Survey of Finland, Special Paper* 49: 153–161.

Vohla C, Kõiv M, Bavor HJ, Chazarenc, F, Mander, Ü (2011) Filter materials for phosphorus removal from wastewater in treatment wetlands—A review. *Ecological Engineering* 37(1): 70–89.

Voigt C, Marushchak ME, Lamprecht RE, Jackowicz-Korczyński M, Lindgren A, Mastepanov M, Granlund L, Christensen TR, Tahvanainen T, Martikainen PJ, Biasi C (2017) Increased nitrous oxide emissions from Arctic peatlands after permafrost thaw. *Proceedings of the National Academy of Sciences*: 201702902.

Vymazal J (2011) Constructed wetlands for wastewater treatment: five decades of experience. *Environmental Science & Technology* 45: 61–69.

Vymazal J (2013) Emergent plants used in free water surface constructed wetlands: A review. *Ecological Engineering* 61: 582–592.

Vymazal J (2014) Constructed wetlands for treatment of industrial wastewaters: A review. *Ecological Engineering* 73: 724–751.

Vymazal J, Březinová T (2015) The use of constructed wetlands for removal of pesticides from agricultural runoff and drainage: A review. *Environment International* 75: 11–20.

Waddington JM, Roulet NT, Swanson RV (1996) Water table control of CH_4 emission enhancement by vascular plants in boreal peatlands. *Journal of Geophysical Research* 101(D17): 22775–22785.

Waddington J, Day S (2007) Methane emissions from a peatland following restoration. *Journal of Geophysical Research-Biogeosciences* 112: G03018.

Waddington JM, Plach J, Cagampan JP, Lucchese M, Strack M (2009) Reducing the carbon footprint of Canadian peat extraction and restoration. *AMBIO: a Journal of the Human Environment* 38(4): 194–200.

Wahlroos O, Valkama P, Mäkinen E, Ojala A, Vasander H, Väänänen VM, Halonen A, Lindén L, Nummi P, Ahponen H, Lahti K (2015) Urban wetland parks in Finland: improving water quality and creating endangered habitats. *International Journal of Biodiversity Science, Ecosystem Services & Management* 11(1): 46–60.

Wang T, Wei H, Hu Z, Chai H, Zhao H (2017) Isolation and identification of a heterotrophic nitrifying and aerobic denitrifying strain and its denitrification characteristics. *Acta Scientiae Circumstantiae* 37: 945–953.

Weber KP (2016) Microbial Community Assessment in Wetlands for Water Pollution Control: Past, Present, and Future Outlook. *Water* 8(11): 503.

Welsh A, Chee-Sanford JC, Connor LM, Löffler FE, Sanford RA (2014) Refined NrfA phylogeny improves PCR-based *nrfA* gene detection. *Applied and environmental microbiology* 80(7): 2110–2119.

Wickham H (2009) ggplot2: Elegant Graphics for Data Analysis. USA, Springer.

Wilson D, Farrell CA, Muller C, Hepp S, Renou-Wilson F (2013) Rewetted industrial cutaway peatlands in western Ireland: A prime location for climate change mitigation? *Mires and Peat* 11(1): 1–22.

Wrage N, Velthof GL, van Beusichem ML, Oenema O (2001) Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biology and Biochemistry* 33: 1723–1732.

Xiong JB, Guo G, Mahmood Q, Yue M (2011) Nitrogen removal from secondary effluent by using integrated constructed wetland system. *Ecological Engineering* 37: 659–662.

Xiong JB, Mahmood Q (2010) Adsorptive removal of phosphate from aqueous media by peat. *Desalination* 259: 59–64.

Yang JS, Liu JS, Wang JD, Yu JB, Sun ZG, Li XH (2006). Emissions of CH_4 and N_2O from a wetland in the Sanjiang Plain. *Journal of Plant Ecology* 30: 432–440.

Yrjälä K, Tuomivirta T, Juottonen H, Putkinen A, Lappi K, Tuittila ES, Penttilä T, Minkkinen K, Laine J, Peltoniemi K, Fritze H (2011) CH_4 production and oxidation processes in a boreal fen ecosystem after long-term water table drawdown. *Global change biology* 17: 1311–1320.

Zehr JP, Jenkins BD, Short SM, Steward GF (2003) Nitrogenase gene diversity and microbial community structure: a cross-system comparison. *Environmental Microbiology* 5: 539–554

Zhang BY, Zheng JS, Sharp RG (2010) Phytoremediation in engineered wetlands: mechanisms and applications. *Procedia Environmental Sciences* 2: 1315–1325.

Zhang QL, Liu Y, Ai GM, Miao LL, Zheng HY, Liu ZP (2012) The characteristics of a novel heterotrophic nitrification–aerobic denitrification bacterium, *Bacillus methylotrophicus strain L7*. *Bioresource Technology* 108: 35–44.

Zhang S, Sun X, Fan Y, Qiu T, Gao M, Wang X (2017) Heterotrophic nitrification and aerobic denitrification by *Diaphorobacter polyhydroxybutyrativorans SL-205* using poly (3-hydroxybutyrate-co-3-hydroxyvalerate) as the sole carbon source. *Bioresource Technology* 241: 500–507.

Zhu B, van Dijk G, Fritz C, Smolders AJ, Pol A, Jetten MS, Ettwig KF (2012) Anaerobic oxidization of methane in a minerotrophic peatland: Enrichment of nitrite-dependent methane-oxidizing bacteria. *Applied and Environmental Microbiology* 78(24): 8657–8665.

Zou Y-Y, Zhang Y, Li, M-Z, Mei R-W, Wei Y-F, Ding L-X (2016) Isolation and identification of a heterotrophic nitrification-aerobic denitrification bacterium and its denitrification ability. *China Environmental Science* 36(3): 887–893.

Zumft WG (1997) Cell biology and molecular basis of denitrification. *Microbiology and molecular biology reviews* 61(4): 533–616.

SUMMARY IN ESTONIAN

Inimtegevuse mõju turbaalade mikrobioomile ja kasvuhoonegaaside emissioonile

Maismaa pindalast on umbes 3% kaetud turbaaladega ja need kõrge orgaanilise aine sisaldusega ökosüsteemid kätkevad endas ⅓ kogu maailma mullasüsinikust. Seda maailma kõige enamlevinud märgalade tüüpi esineb alates troopikast kuni boreaalsete ja arktiliste piirkondadeni. Kuigi turbaalasid on peetud tähtsaks eelkõige põhjapoolkera parasvöötme kontekstis, siis hiljutised avastused näitavad, et troopilistes regioonides leidub võrreldes varasemate hinnangutega kuni kolm korda enam turvast. Lisaks sellele on täheldatud igikeltsa kiiret ja ulatuslikku sulamist arktilistel turbaaladel, mis on toonud aktiivsetesse ringetesse aina enam varasemalt pinnasesse salvestunud süsinikku ja lämmastikku. Seega mängivad turbaalade süsiniku- ja lämmastikuringe olulist rolli keskkondlikust, ökoloogilisest ja majanduslikust vaatevinklist. Seda eriti arvestades üha suurenevat kliimamuutuste ja inimmõjutuste survet turbaaladele. Need häiringud toovad kaasa muutused mikrobioloogilises koosluses ja biogeokeemilistes protsessides, mis omakorda mõjutavad otseselt ka kasvuhoonegaaside (metaan (CH_4), dilämmastikoksiid (N_2O)) emissioone.

Käesoleva töö eesmärk oli uurida erinevate majandusmeetmete mõju turbaalade mikrobioomile ja kasvuhoonegaaside emissioonile. Välitöid tehti parasvöötmelise Eesti ja troopilise Prantsuse Guajaana turbaaladel. Kaheaastasel eksperimendil Lavassaare (Pärnumaa) mahajäetud freesturba tootmisalal hinnati päiderooga kultiveerimise ja väetamise mõju turba mikroobikooslusele ja CH_4 tootmise potentsiaalile. Troopikas asuval uurimisalal hinnati kuivenduse mõju turbaala mikroobikooslusele, lämmastikuringe protsesside potentsiaalile ja kasvuhoonegaaside emissioonile. Lisaks tehti 87 uuringul põhinev metaanalüüs põhjapoolkera laiuskraadidel 40° kuni 70°N asuvatest turbaaladest võttes arvesse 186 uurimisala CH_4 emissioone erinevatest riikidest vastavalt turbaala tüüpidele ja inimmõjudele. Lisaks sellele koostati ülevaade turba ja turbaalade kasutamisest erinevate reoainete puhastamiseks veest.

Põhjapoolkera parasvöötme looduslikud turbaalad on olulisteks CH_4 voogude allikaks atmosfääri, kuigi CH_4 emissioonid võivad olla väga varieeruvad sõltuvalt peamiselt järgmistest faktoritest: turbaala tüüp, turba pH, veetase ja õhutemperatuur. Turbaalade kuivendamisel väheneb CH_4 emissioon keskmiselt 84%. Rabades on kuivenduse mõju CH_4 emissioonile väiksem kui madalsoodes.

Kuivendamise protsess avaldab suurt mõju ka troopilise turbaala gaasiemissioonidele ning seega ka mikroobikooslusele. Kuigi CH_4 emissioon kahanes kuivendamisel, siis N_2O voog atmosfääri suurenes. Samuti oli väiksem potentsiaalne molekulaarse lämmastiku (N_2) emissioon ülemisest 10 cm kihist kuivendatud alal võrreldes loodusliku alaga. Lisaks muutustele gaasiemissioonides, suurenes kuivendamise tulemusel turbas nende bakterite ja arhede rühmade esindajate osakaal, keda seostatakse enam taimse biomassi lagundamisega: hõimkonnad *Actinobacteria*, *Firmicutes*, *Crenarchaeota* ja klass

Thermoplasmata hõimkonnast *Euryarchaeota*. Troopilise turbaala kuivendamine muutis peale bakterite ja arhede arvukuste ka oluliselt selliste geenide arvukusi, mis kodeerivad erinevaid lämmastikuringe protsesse läbiviivaid ensüüme. Looduslikku ala iseloomustas suurem nitriti redutseerimise (*nirK*, *nirS*), N_2O redutseerimise (*nosZ*) ja N_2 fikseerimise (*nifH*) potentsiaal, kuid nitrifikatsiooni (arhede *amoA*) potentsiaal oli seal võrreldes kuivendatud turbaalaga väiksem. DNRA (dissimilatoorne nitraadi redutseerimine ammooniumiks) protsessi potentsiaal ilmnes ainult looduslikul turbaalal. Kui looduslikul alal oli N_2O emissioon seotud peamiselt *nirS* geeni omavate mikroorganismidega ja DNRA protsessiga ning N_2O redutseerimist kontrollisid *nosZ* klaad I geeni omavad mikroobid, siis kuivendatud alal olid N_2O redutseerijateks pigem *nosZ* klaad II geeni kandvad organismid.

Põhjapoolkera parasvöötme häiritud turbaalade taassoostamine suurendab CH_4 emissiooni keskmiselt 46% võrreldes taastamiseelsete CH_4 voogudega. Mahajäetud turbakaevandusalade korrastamine päideroo (*Phalaris arundinacea*) kasvatamisega bioenergia tootmise eesmärgil vähendas CH_4 emissiooni, kuigi metanogeenide arvukus turba erinevates kihtides jäi umbes samale tasemele või siis isegi suurenes katseperioodi jooksul. Muutused toimusid ka turba füüsikalis-keemilistes omadustes ning päiderooga kultiveerimise tulemusel bakterikoosluse arvukus suurenes. Kuigi metanogeenide arvukus arhede koosluses oli üldiselt madal, siis suurima CH_4 tootmise potentsiaaliga olid turba sügavamad kihid. Kui päiderooga kultiveerimata mahajäetud turbaalal mõjutasid CH_4 emissiooni peamiselt sügavamate kihtide metanogeenid, siis päiderooga kultiveerimise puhul võib oletada metanotroofide tähtsuse tõusu turba süsinikuringes.

Turbaalasid on sooderikastes piirkondades võimalik kasutada reovee puhastamiseks, et kaitsta veekogude ökosüsteeme reoainete eest. Turbaalad võivad olla madalate energia- ja hoolduskuludega efektiivsed looduslikud reoveepuhastid ning selliseid turbaalapuhasteid on käsitletud sobiva meetmena üleliigse lämmastiku, fosfori, hõljuvaine, väävli ning erinevate metallide eemaldamiseks. Kindlasti on turbaalapuhastites oluline roll ka taimedel, mis võivad olla läbi erinevate mehhanismide efektiivseteks reoainete eemaldajateks.

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Espenberg M, Truu M, Truu J, Maddison M, Nõlvak H, Järveoja J, Mander Ü (2016) Impact of reed canary grass cultivation and mineral fertilisation on the microbial abundance and genetic potential for methane production in residual peat of an abandoned peat extraction area. *PLoS ONE* 11(9): e0163864.

Kasak K, Mõtlep R, Truu M, Truu J, Kõiv-Vainik M, **Espenberg M**, Paiste P, Kirsimäe K, Mander Ü (2016) Hydrated oil shale ash mitigates greenhouse gas emissions from horizontal subsurface flow filters for wastewater treatment. *Water, Air, & Soil Pollution* 227(9): 320.

Nõlvak H, Truu M, Kanger K, Tampere M, **Espenberg M**, Loit E, Raave H, Truu J (2016) Inorganic and organic fertilizers impact the abundance and proportion of antibiotic resistance and integron-integrase genes in agricultural grassland soil. *Science of the Total Environment* 562: 678–689.

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Espenberg M, Truu M, Truu J, Maddison M, Nõlvak H, Järveoja J, Mander Ü (2016) Impact of reed canary grass cultivation and mineral fertilisation on the microbial abundance and genetic potential for methane production in residual peat of an abandoned peat extraction area. *PLoS ONE* 11(9): e0163864.

Kasak K, Mõtlep R, Truu M, Truu J, Kõiv-Vainik M, **Espenberg M**, Paiste P, Kirsimäe K, Mander Ü (2016) Hydrated oil shale ash mitigates greenhouse gas emissions from horizontal subsurface flow filters for wastewater treatment. *Water, Air, & Soil Pollution* 227(9): 320.

Nõlvak H, Truu M, Kanger K, Tampere M, **Espenberg M**, Loit E, Raave H, Truu J (2016) Inorganic and organic fertilizers impact the abundance and proportion of antibiotic resistance and integron-integrase genes in agricultural grassland soil. *Science of the Total Environment* 562: 678–689.

Truu J, Truu M, **Espenberg M**, Nõlvak H, Juhanson J (2015) Phytoremediation and plant-assisted bioremediation in soil and treatment wetlands: a review. *The Open Biotechnology Journal* 9: 85–92.

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