**Dissertationes Forestales 252**

# *Sphagnum*-associated methanotrophs – a resilient $CH_4$ biofilter in pristine and disturbed peatlands

Anuliina Putkinen

Faculty of Biological and Environmental Sciences
The Doctoral Programme in Microbiology and Biotechnology
University of Helsinki

Academic dissertation

To be presented, with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki, for public examination in Auditorium 1, Viikki Infocenter Korona (Viikinkaari 11, Helsinki) on April 20th 2018, at 12 o'clock noon.

*Title of the dissertation: Sphagnum*-associated methanotrophs – a resilient $CH_4$ biofilter in pristine and disturbed peatlands

*Author*: Anuliina Putkinen

*Dissertationes Forestales 252*

https://doi.org/10.14214/df.252


*Thesis Supervisors:*
Professor Hannu Fritze
Natural Resources Institute Finland (Luke)

Professor Eeva-Stiina Tuittila
School of Forest Sciences, University of Eastern Finland

Docent Kim Yrjälä
Department of Forest Sciences, University of Helsinki

*Pre-examiners:*
Professor Mette Svenning
Department of Arctic and Marine Biology, UiT – The Arctic University of Norway

Professor Jaak Truu
Institute of Ecology and Earth Sciences, University of Tartu

*Opponent:*
Head of Laboratory, Dr. Svetlana Dedysh
Laboratory of Wetland Microbiology, Winogradsky Institute of Microbiology, Russian Academy of Sciences

ISSN 1795-7389 (online)
ISBN 978-951-651-594-9 (pdf)

ISSN 2323-9220 (print)
ISBN 978-951-651-595-6 (paperback)

*Publishers:*
Finnish Society of Forest Science
Faculty of Agriculture and Forestry at the University of Helsinki
School of Forest Sciences at the University of Eastern Finland

*Editorial Office:*
Finnish Society of Forest Science, Dissertationes Forestales
Viikinkaari 6, 00790 Helsinki
http://www.dissertationesforestales.fi

**Putkinen A.** (2018). *Sphagnum*-associated methanotrophs – a resilient $CH_4$ biofilter in pristine and disturbed peatlands. Dissertationes Forestales 252. 61 p. Available at https://doi.org/10.14214/df.252

## ABSTRACT

Boreal peatlands are highly important sinks for carbon (C). This function is enabled largely by one peat-forming plant, the *Sphagnum* moss. In addition to slowing the decomposition by gradually creating ombrotrophic conditions, it gives a shelter for the organisms mitigating the emissions of methane ($CH_4$) – an effective greenhouse gas formed in submerged, anoxic peat layers. These organisms, methane oxidizing bacteria (methanotrophs, MOB), inhabit the dead, water-filled hyaline cells of the *Sphagnum* and provide the plant carbon dioxide ($CO_2$) derived from the $CH_4$ oxidation. While several studies have confirmed the presence of *Sphagnum*-associated methanotrophs (SAM), it is still unclear how dependent they are on the mosses and how environmental conditions affect their community composition and activity.

This thesis evaluated SAM dynamics in the different stages of peatland development on both pristine and disturbed areas. Studies were based mainly on molecular methods, targeting the MOB-specific *pmoA* gene, and laboratory incubations, including stable isotope probing.

In the first study, the connection between the SAM and the mosses was assessed by testing whether the SAM will disperse through the water phase. This trait, considered to represent a facultative symbiosis, was demonstrated in two experiments. In the field, mosses inactive in $CH_4$ oxidation were transplanted next to active ones. Within a month, SAM communities of the neighboring mosses become more similar. The water-based colonization was further confirmed by bathing inactive mosses in flark pore water that showed high $CH_4$ oxidation activity. Within just 11 h, activity was induced and the SAM abundance significantly increased in the treated mosses.

The other two studies revealed similar SAM community composition patterns on a pristine chronosequence and on a gradient of re-vegetating cutover peatlands. Instead of the *Sphagnum* species, the general environmental conditions seemed to control the SAM community composition. Different types of SAM seemed to have their preferred environmental niches, with the type Ia MOB present and active especially in the young succession stages and the type II MOB in the older, hydrologically more stable stages. Despite the community differences, the potential $CH_4$ oxidation did not differ along the gradients, suggesting functional redundancy. Only some drier bog samples did not show any detectable $CH_4$ oxidation, demonstrating the regulatory role of the water table level on the SAM activity. The peat layers of the cutover gradient showed similar MOB community patterns but the potential $CH_4$ oxidation increased with succession.

The ability to disperse through the water provides a recovery mechanism from disturbances such as droughts, which are predicted to increase with climate warming. In addition, the diversity and functional redundancy of the SAM communities enhance the resilience of this important $CH_4$ biofilter formed by the living *Sphagnum* mosses. The potential SAM activity in the mosses of the youngest cutover site promotes the *Sphagnum* transplantation practice as a tool to not only enhance the re-vegetation process, but also to mitigate the $CH_4$ emissions formed in the rewetting and restoration of disturbed peatlands.

Keywords: plant-microbe interactions, $CH_4$ oxidation, microbial community analysis, *pmoA* microarray, ecosystem restoration, greenhouse gas emissions

## ACKNOWLEDGEMENTS

This thesis was funded mainly by the Maj and Tor Nessling Foundation. Grants were received also from the Finnish Society of Forest Science, from the Niemi Foundation, from the Finnish Concordia Fund, from the University of Helsinki and, for the first, restoration-themed research plan, from the University of Helsinki Science Foundation. Finalization of the thesis was conducted partly with the support from the Academy of Finland Center of Excellence in Atmospheric Science. The thesis work was conducted mainly at the Natural Resources Institute Finland (Luke), which provided excellent facilities for the laboratory analyses.

Many people supported my efforts throughout these years. Firstly, I'm highly grateful for the help and guidance offered by my supervisors, Hannu Fritze, Eeva-Stiina Tuittila and Kim Yrjälä. Hannu, thank you for the position in your Nessling-funded project and for allowing me to continue the previously started restoration-themed studies as well. Evu, this work would not have been possible without your expertise in peatland ecology and statistics, and without your eagerness to carry out the microbial study of the Aitoneva sites. Kim, your role got smaller after my topic focused on the SAM, but nevertheless, I appreciate your support and endless enthusiasm for research. My thesis continued the SAM-related work of Tuula Larmola – thank you, Tuula, for being so helpful during the whole project. Thanks also to Tero Tuomivirta for the molecular biology expertise and particularly in helping with the SIP analysis. Levente Bodrossy and Henri Siljanen enabled the *pmoA* microarray analysis – thanks especially to Henri for the hands-on contribution. Sirpa Tiikkainen and Minna Oksanen, your skills in the lab were invaluable to me. Markus Hartman, thank you for editing the language of the extended summary and of the last publication, and for the useful comments on their content. Thank you also to everyone else, who made valid contributions to the studies conducted during this thesis process (not all included in the thesis).

Sarah Butcher kindly agreed to serve as the custos in my defense. Thank you for being so supportive during these last months and for battling the thesis bureaucracy with me. Thank you to the thesis committee members, Kristina Lindström, Taina Pennanen and the late Kielo Haahtela, for your valuable advice. My sincere thanks belong also to Svetlana Dedysh, for agreeing to act as the opponent in my defense, to Mette Svenning and Jaak Truu, for conducting the pre-examination of this thesis, and to Rauni Strömmer, for agreeing to be a member of the thesis grading committee.

In addition, I'm particularly thankful to the following persons who had a major impact on this thesis: Firstly, my current project leader Mari Pihlatie: a position in your group returned my motivation in research at a time when I needed it the most. Thank you for your invaluable support in the finalization of this thesis. Secondly, Meeri Pearson: Without you, this thesis might have been finished long ago, but it would also have been covered with fairytales. I cannot tell you how grateful I am for the information you provided, and for your friendship. Last but not least, my dear friend and mentor, Heli Juottonen: Starting from my Master's thesis, you have been the one that I have bombarded with stupid questions. Thank you for your help and patience.

Thank you also for the much needed peer support provided by my other colleagues. Mirja, Timo, Shinjini, Mirkka and Heli, I hope we will play 'Lupaus' together even when we have retired. Annu, you were an awesome office mate during our years in Luke. Krista, thanks for always being helpful when I walked through your door. Anu, Minna, Outi and other Viikki microbiologists, thank you for the great company. Thanks also to the whole 'forest-methane group' and its collaborators; I'm very happy to be able to continue working with you all.

Finally, my warmest thanks to my oldest friends Tiia, Niina and Johanna, to my dear family, who has successfully kept taking my thoughts away from the thesis, and to my closest supporter, Kimmo, for reminding me that a little bit of 'sisu' goes a long way.

Apparently, every story has an ending – even this thesis project. The journey was *a bit* different from what I envisioned, with a few unnecessary bumps on the road. With all the more reason, I could not be happier for finally reaching the finish line.

Vantaa, March 2018

Anuliina Putkinen

## LIST OF ORIGINAL PUBLICATIONS

I. Putkinen A., Larmola T., Tuomivirta T., Siljanen H.M.P., Bodrossy L., Tuittila E.-S., Fritze H. (2012). Water dispersal of methanotrophic bacteria maintains functional methane oxidation in *Sphagnum* mosses. Frontiers in Microbiology 3: 15. http://dx.doi.org/10.3389/fmicb.2012.00015

II. Putkinen A., Larmola T., Tuomivirta T., Siljanen H.M.P., Bodrossy L., Tuittila E.-S., Fritze H. (2014). Peatland succession induces a shift in the community composition of *Sphagnum*-associated active methanotrophs. FEMS Microbiology Ecology 88: 596–611. http://dx.doi.org/10.1111/1574-6941.12327

III. Putkinen A., Tuittila E.-S., Siljanen H.M.P., Bodrossy L., Fritze H. (2018). Recovery of methane turnover and the associated microbial communities in restored cutover peatlands is strongly linked with increasing *Sphagnum* abundance. Soil Biology and Biochemistry 116: 110-119. http://dx.doi.org/10.1016/j.soilbio.2017.10.005



## THE AUTHOR'S CONTRIBUTION

I. A.P. conducted the microarray analysis of the MOB communities with H.S. and the data analysis with E.-S.T., interpreted the results and wrote the paper. She was the corresponding author.

II. A.P. participated in the design of the study, was responsible for the incubations and the molecular analysis (microarray with H.S.), conducted the data analysis with E.-S.T., interpreted the results and wrote the paper. She was the corresponding author.

III. A.P. designed the study and took the samples with E.-S.T., measured the potential activities, conducted the *mcrA*-T-RFLP, conducted the microarray analysis with H.S. and the data analysis with E.-S.T., interpreted the results and wrote the paper. She was the corresponding author.

# TABLE OF CONTENTS

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AN | Aitoneva peat extraction site |
| ANOSIM | analysis of similarities |
| ANOVA | analysis of variance |
| C | carbon |
| CCA | canonical correspondence analysis |
| $CH_4$ | methane |
| CLSM | confocal laser scanning microscopy |
| $CO_2$ | carbon dioxide |
| DGGE | denaturing gradient gel electrophoresis |
| DNA | deoxyribonucleic acid |
| FISH | fluorescent in situ hybridization |
| GC | gas chromatograph |
| HPLC-PDA | high-performance liquid chromatography photodiode array |
| *mcrA* | gene coding for the α -subunit of the methyl coenzyme M reductase |
| MOB | methane oxidizing bacteria, methanotroph |
| MOP | methane oxidation potential |
| MPP | methane production potential |
| $N_2$ | dinitrogen |
| *nifH* | gene coding for the iron-protein component of the nitrogenase complex |
| NMDS | non-metric multidimensional scaling |
| PCA | principal component analysis |
| PCR | polymerase chain reaction |
| pMMO | particulate methane monooxygenase |
| *pmoA* | gene coding for the α-subunit of the particulate methane monooxygenase |
| pRDA | partial redundancy analysis |
| qPCR | polymerase chain reaction |
| RDA | redundancy analysis |
| RFLP | restriction fragment length polymorphism |
| RNA | ribonucleic acid |
| SAM | *Sphagnum*-associated methanotroph |
| SIP | stable isotope probing |
| sMMO | soluble methane monooxygenase |
| SSCP | single-strand conformation polymorphism |
| T-RF | terminal restriction fragment |
| T-RFLP | terminal restriction fragment length polymorphism |
| Tukey’s HSD | Tukey's honest significance test |
| WT | water table |
| 16S rDNA | 16S ribosomal DNA |

# 1 INTRODUCTION

## 1.1 Methane as a greenhouse gas

Methane ($CH_4$) is a greenhouse gas with a 28-32 times stronger warming potential than carbon dioxide ($CO_2$) over a hundred-year time scale (IPCC, Myhre et al. 2013; Etminan et al. 2016). It is formed at least in three different processes: biologically mainly through the anaerobic degradation of organic matter, thermogenically during the very slow geological formation of fossil fuels and pyrogenically during incomplete combustion of organic matter (IPCC, Ciais et al. 2013). Within these processes, $CH_4$ sources can be divided into natural and anthropogenic sources with the former including for example wetlands, freshwater sediments, termite guts and wildfires, and the latter for example rice cultivation, farming of ruminant animals and the use of fossil fuels. Recently, $CH_4$ emissions have been measured from living vegetation, including trees (Keppler et al. 2006; Machacova et al. 2016; Pangala et al. 2017). Although the mechanisms behind these emissions are still uncertain, they have already changed the concept of exclusively anaerobic biological $CH_4$ formation (Lenhart et al. 2012). The opposite process to $CH_4$ production, oxidation of the atmospheric $CH_4$, takes place either by chemical reactions with the OH-radicals mainly in the troposphere (90 % of the total sink for the atmospheric $CH_4$, Ciais et al. 2013) or by the actions of $CH_4$ oxidizing bacteria (MOB) mainly in the upland soil (Le Mer and Roger 2001). In addition, MOB are able to oxidize a large part of the soil-derived $CH_4$ even before it reaches the atmosphere (e.g. Whalen 2005).

Between 2000 and 2009, the annual total $CH_4$ emissions were in the range of 553 to 678 Tg $CH_4$ $y^{-1}$ with an almost equal distribution between the natural and anthropogenic sources (IPCC, Ciais et al. 2013). Wetland-related biogenic emissions and emissions originating from agriculture and waste were the largest contributors, respectively. Since the pre-industrial times (before 1750), the atmospheric $CH_4$ concentration has risen from 0.72 ppm to 1.8 ppm – mainly due to anthropogenic activities (IPCC, Hartmann et al. 2013).

## 1.2 Microbiological methane cycle

### *1.2.1 Methane producing archaea, the methanogens*

Based on current knowledge, most of the biologically formed $CH_4$ is derived from a specific type of microbes, the $CH_4$ producing archaea, i.e. the methanogens - although $CH_4$ has been recently shown to be formed also within the aerobic metabolism of saprotrophic fungi (Lenhart et al. 2012) and also in connection to $N_2$ fixation (Zheng et al. 2018). Within the domain Archaea, methanogens are found in the phylum Euryarchaeota and within seven different orders: *Methanomicrobiales*, *Methanobacteriales*, *Methanosarcinales*, *Methanococcales*, *Methanocellales*, *Methanopyrales* and *Methanomassiliicoccales* (Nazaries et al. 2013; Lang et al., 2015). In addition, recent metagenomics studies indicate that methanogens can be found within the two newly discovered phyla, Bathyarchaeota (Evans et al., 2015) and Verstraetearchaeota (Vanwonterghem et al. 2016). Currently known methanogens are a phylogenetically relatively coherent group, but instead of the universal 16S rDNA, their analysis is often based on the functional *mcrA* gene coding for the α-subunit of the methyl coenzyme M reductase (Luton et al. 2002; Steinberg and Regan 2008), which

allows the exclusion of closely related non-methanogenic archaea. Archaeal methanogenesis is a strictly anaerobic process and thus methanogens are found especially in water-saturated environments, such as wetlands, sediments and rice fields. Most of them are mesophiles, although extremophilic strains have been detected in the geothermal environments (Nazaries et al. 2013). Methanogens cooperate closely with other microbes, including the homoacetogenic and the fermentative bacteria, preceding them in the chain of organic matter degradation. Secondary fermenters, or syntrophs, provide the substrates, such as $H_2$, used by the hydrogenotrophic methanogens to reduce $CO_2$ to $CH_4$, and acetate, used by the acetoclastic methanogens (Schink 1997). Most methanogens are hydrogenotrophs and indications of this trait have been found even in the genus *Methanosaeta*, which was long thought to contain only obligate acetogens (Rotaru et al. 2014). The *Methanosarcinales* differ from the former with their versatility, as some related species are capable of using both of the mentioned pathways and even other C substrates such as methanol and methylamines (Costa and Leigh, 2014). The recently characterized phylum, *Methanomassiliicoccales*, is proposed to contain obligate methylotrophs that need $H_2$ for the $CH_4$ production (Lang et al. 2015).

### *1.2.2 Methane oxidizing bacteria, the methanotrophs*

Methane oxidizing bacteria (MOB) are a functional guild of microorganisms capable of using $CH_4$ as a sole source of carbon (C) and energy. The currently known aerobic MOB encompass a diverse group of microbes from three different phyla. Within the two larger MOB groups, Alphaproteobacteria includes five MOB genera and Gammaproteobacteria 18 MOB genera that exist as cultured isolates (Knief, 2015, Table 1). In addition, one alpha- (Pratcher et al. 2018) and two gammaproteobacterial (Stoecker et al. 2006; Vigliotta et al. 2007) genera have been proposed despite the lack of successful isolation. A decade ago, MOB were discovered also in the phylum Verrucomicrobia (Dunfield et al. 2007; Pol et al. 2007; Islam et al. 2008), which now consist of two genera (Op den Camp et al. 2009; van Teeseling et al. 2014).

Currently, the classification of MOB is largely based on the analysis of two phylogenetically congruent marker genes, the universal 16S rRNA, found in all prokaryotes, and the MOB specific *pmoA*, coding for the α-subunit of the particulate methane monooxygenase (pMMO), that converts $CH_4$ to methanol (McDonald et al. 2008). As an exception, some MOB genera have only the soluble form of the methane monooxygenase (sMMO) and their analysis is based on the *mmoX* gene instead of the *pmoA* (Dedysh et al. 2005, Vorobev et al. 2011, Liebner and Svenning 2013). In addition to phylogenetic differences, the MOB have some distinct physiological and morphological traits that have been traditionally used to classify them into two main groups: type I and type II, which are known to correspond well to gamma- and alphaproteobacteria, respectively. With increasing knowledge on the diversity of the traits within these two MOB types, fewer traits are now considered specific to either one of them (Knief, 2015). The main distinctive feature is still the pathway for carbon assimilation: Type I use the ribulose monophosphate pathway and type II use the serine cycle (Hanson and Hanson 1996). Other features quite specific for either group include formation of internal membranes set as vesicular discs (type I) or as pairs aligned to the cell periphery (type II) and the differences in the fatty acid composition of their cell membranes. The ability to fix nitrogen ($N_2$) and to form resting stages such as cysts is not limited to but still more pronounced in the type II MOB (Hanson and Hanson 1996; Knief 2015). Based on both phylogenetic and physiological differences, the type I MOB have been

Table 1. Current taxonomic grouping and basic features of the aerobic methane oxidizing bacteria (MOB) [according to Knief (2015) and the references therein, and Pratcher et al. (2018)].

| Phylum | Family | Genera | type | *pmoA* | *mmoX* | facultative[b] |
|---|---|---|---|---|---|---|
| Alphaproteobacteria | *Methylocystaceae* | *Methylocystis* | II | + | + | + |
| | | *Methylosinus* | II | + | + | - |
| | *Beijerinckiaceae* | *Methylocapsa* | II | + | - | + |
| | | *Methylocella* | II | - | + | + |
| | | *Methyloferula* | II | - | + | - |
| | | *Ca.* Methyloaffinis[a] | na | + | - | - |
| Gammaproteobacteria | *Methylococcaceae* | *Methylobacter* | Ia | + | - | - |
| | | *Methyloglobulus* | Ia | + | - | - |
| | | *Methylomarinum* | Ia | + | - | - |
| | | *Methylomicrobium* | Ia | + | + | - |
| | | *Methylomonas* | Ia | + | - | - |
| | | *Methyloprofundus* | Ia | + | - | - |
| | | *Methylosarcina* | Ia | + | - | - |
| | | *Methylosoma* | Ia | + | - | - |
| | | *Methylosphaera* | Ia | + | - | - |
| | | *Methylovulum* | Ia | + | + | - |
| | | *Methylocaldum* | Ib | + | + | - |
| | | *Methylococcus* | Ib | + | + | - |
| | | *Methylogaea* | Ib | + | - | - |
| | | *Methyloparacoccus* | Ib | + | - | - |
| | | *Methylomagnum* | Ib | + | + | - |
| | | *Ca.* Clonothrix[a] | na | + | - | - |
| | *Methylothermaceae* | *Methylohalobius* | 1c | + | - | - |
| | | *Methylomarinovum* | 1c | + | - | - |
| | | *Methylothermus* | 1c | + | - | - |
| | *Crenotrichaceae* | *Ca.* Crenothrix[a] | na | + | - | + |
| Verrucomicrobia | *Methylacidiphilaceae* | *Methylacidiphilum* | na | + | - | - |
| | | *Methylacidimicrobium* | na | + | - | - |

na = not applicable, + = detected at least in some strains, - = not detected or analyzed

[a] *Candidatus* genus, no cultivated strains yet

[b] able to use other C sources in addition to $CH_4$

further split in two groups, type Ia and Ib (formely known as type X) (Table 1). Some publications have included also the group of type Ic which has included either some uncultivated MOB taxa and the *amoA* sequence of *Nitrosococcus* (e.g. Dumont et al. 2014) or the family *Methylothermaceae* (e.g. Knief, 2015). In the MOB community analysis within this thesis, the classification to the MOB types Ia, Ib and II is used as described in Knief (2015) (Table 1).

As can be expected from their high diversity, the MOB are ubiquitous in nature and can be found in various different environments. Habitats with high moisture levels (e.g. wetlands, lakes, rice fields) and therefore with high *in situ* $CH_4$ production, favor MOB adapted to utilize $CH_4$ in high $CH_4$ concentrations (i.e. low-affinity MOB, Bender and Conrad 1992) – here the MOB often live on the anoxic-oxic interphase where they have access to both the $CH_4$ from below and to the oxygen from above. In contrast, the dryer conditions (e.g. upland forests) favor MOB that are able to oxidize $CH_4$ in low, atmospheric concentrations (high-affinity MOB, Bender and Conrad, 1992; Pratcher et al. 2018). At least some *Methylocystis* and *Methylosinus* strains harbor an additional pMMO enzyme (pMMO2), which enables them to use $CH_4$ also in the low concentrations (Yimga et al. 2003). Most MOB prefer moderate pH levels between 6 to 8 and temperatures in the range of 20-40 °C (Whittenbury et al. 1970). The Verrucomicrobial MOB have been detected mostly in geothermal areas in temperatures as high as 65 °C and in a pH as low as 0.8 (Op den Camp et al. 2009) but these have also been detected in lower temperatures (Sharp et al. 2014). In addition to $CH_4$, some MOB (e.g. some *Methylocella*, *Methylocapsa* and *Methylocystis* strains) are able to use other substrates, such as acetate, methanol and $CO_2$, for C and/or energy (Theisen et al. 2005; Im et al. 2011; Belova et al. 2011).

In addition to aerobic methanotrophs, a diverse group consisting of both bacteria and archaea oxidize $CH_4$ in anoxic conditions by using electron acceptors such as sulfate, nitrate or iron (e.g. Michaelis et al. 2002; Raghoebarsing et al. 2006; Ettwig et al. 2016). These anaerobic $CH_4$ oxidizers (ANME) are common in the marine environments but may also be found in freshwater environments, such as lake sediments (e.g. Weber et al. 2017) and peatlands (e.g. Gupta et al. 2013; Shi et al. 2017).

### 1.3 The peatland ecosystem

Peatlands are organic soil ecosystems characterized by a high water table (WT) level, which leads to anoxic conditions and, consecutively, slow organic matter degradation and to peat formation. They are globally important C storages as over one third of the global soil organic matter is estimated to be stored in the peat layers. At the same time, they are a major source of $CH_4$, formed in the submerged peat layers (Gorham 1991). Most of the peatlands, 346 Mha, are found in the northern boreal or subarctic zones where, in addition to wet conditions, the low temperatures have retarded the degradation processes (Gorham 1991; Yu 2012). The corresponding peatland areas in the tropics are relatively small (approximately 11%, Page et al. 2011), but emit more $CH_4$ than the boreal regions (Turetsky et al. 2014). As the growth of *Sphagnum* mosses is centered to the boreal peatlands (Rydin and Jeglum 2013), this thesis concentrates on those areas.

Typically, boreal peatland development starts from wet depressions that become vegetated by sedges and brown mosses, and later on, by minerotrophic *Sphagnum* moss species. These young, fen-type peatlands receive nutrients both from the groundwater and from the water flowing from the surrounding areas. With an increasing *Sphagnum* cover and

emergence of hummock forming *Sphagnum* species, the peat surface rises and the vegetation loses contact to other water sources except for precipitation – leading to the formation of nutrient-poor and acidic, ombrotrophic bog-type peatlands (Hughes and Barber 2004; Laine et al. 2011; Väliranta et al. 2016; Gałka et al. 2017). Due to changes in the vegetation composition and the accumulation of peat, this fen-bog succession includes a gradient from highly fluctuating water table (WT) to drier, but more stable hydrological conditions (Leppälä et al. 2008; Tuittila et al. 2013). The above transition, together with the changes in peat physicochemical properties (e.g. decreasing pH and C/N), is reflected in C dynamics (Thormann et al. 1999; Kotiaho et al. 2008; Leppälä et al. 2011).

Most of the current boreal peatland areas originated during the early Holocene period, accelerated by the melting glacier waters (MacDonald et al. 2006). However, new peat forming areas are still being initiated for example through the process of post-glacial land uplifting. This phenomenon leads to the formation of peatland chronosequences, which provide unique opportunities to examine the succession-associated abiotic and biotic factors (e.g. Klinger and Short 1996; Leppälä et al. 2008; Tuittila et al. 2013). In addition to the autogenous processes leading to the fen-bog transition, the direction and rate of the succession process may change in the events of more sudden disturbances, such as wildfires (Tuittila et al. 2007; Singer et al., 1996) and is also influenced by the climate. Currently, due to the slower succession, the proportion of the fens is higher in the colder, northern regions than in the south (Väliranta et al., 2017). In the future, climate change induced warming is predicted to affect the boreal peatland succession in two opposite ways, depending on the latitude: While the southern fens may become drier due to increased evapotranspiration, accelerating their development towards bogs, the northern thawing permafrost regions are expected to show a change from the bog-type vegetation towards wetter fens (Johansson et al., 2006; Tahvanainen et al. 2011).

In addition to the C accumulation, peatlands provide other important ecosystem services such as hydrological buffering, high biodiversity and recreation opportunities (Andersen et al. 2017). They are also utilized as areas for forestry and agriculture and for the extraction of peat. These economical uses of peatlands require drainage of the area, which severely disrupts the peatland C cycle and often turns them from C sinks to sources (Maljanen et al. 2010; Wilson et al. 2016). Of the total global peatland area, approximately 4.3% (15 Mha) has been drained (Paavilainen and Päivänen 1995) but the areal variation is large: for example in Finland approximately two thirds of the peatland area have been drained (Vasander, 2003). Restoration of these degraded peatlands is increasingly promoted as a way to for example mitigate C emissions and conserve rare ecosystems (Chimner et al. 2017; Andersen et al. 2017), and the aim is to make these efforts also economically feasible through their inclusion in the C emissions factors (Bonn et al. 2014).

## 1.4 Peatlands as a source of methane

### *1.4.1 Methane turnover in pristine boreal peatlands*

The pristine peatlands are important sinks for $CO_2$ but at same time a major source of $CH_4$. Due to the nature of the $CH_4$ production and oxidation as mainly anaerobic and aerobic processes, respectively, the WT level is one of the main controllers of these functions (Whalen and Reeburgh 2000, Abdalla et al. 2016). Most of the methanogens are sensitive to oxygen and are thus often most active in the more permanently anoxic layers approximately

20 cm below the average WT level, where their substrates (acetate, $H_2/CO_2$) are still in adequate supply (Sundh et al. 1994; Kettunen et al. 1999). However, some methanogens might be more tolerant to the oxygen and may thrive in anoxic microsites even close to the surface (Kotiaho et al. 2010; Angle et al. 2017). Methanotrophs are dependent on the activity of methanogens as providers of their substrate but they also need oxygen and are thus often most active close to the interphase of the anoxic and oxic layers (Sundh et al. 1995; Laine et al. 2012). The composition of the ground vegetation controls both the methanogens and the MOB: *Eriophorum vaginatum* and other aerenchymatous plants provide substrates for the methanogens (Ström et al. 2003; Saarnio et al. 2004) and shuttle $CH_4$ directly to the atmosphere and thus out of reach of the MOB (Tuittila et al. 2000a; Cooper et al. 2014). They also provide oxygen for the $CH_4$ oxidation in the rhizosphere (Popp et al. 2000; Fritz et al. 2011). The *Sphagnum* mosses support the methanogens, and indirectly the MOB, by stabilizing the WT but also exert negative effects for example by producing antimicrobial compounds (see section 1.5). Other controlling factors are temperature and pH (e.g. Dunfield et al. 1993; Dedysh and Panikov 1997; Wagner et al. 2017), and in the case of methanogens, the actions of the other microbes in the degradation chain (Schmidt et al. 2016; Juottonen et al. 2017, see also section 1.2.1) and substrate competition with other anaerobic processes, such as sulfate reduction (e.g. Pester et al. 2010).

Due to the complex interactions between hydrology, climate, vegetation, peat properties (impacting e.g. gas diffusion/ebullition) and microbial activity, the actual $CH_4$ emissions can vary greatly between peatlands and within short and long spatial and temporal scales (Turetsky et al., 2014). In general, owing to their differences in the vegetation and hydrology, fens emit more $CH_4$ than bogs (Turetsky et al. 2014; Abdalla et al. 2016). Climate warming is expected to be relatively rapid in the northern latitudes, which may lead to an increase in the $CH_4$ emissions induced by the melting permafrost (Schuur et al. 2015). In addition, a predicted increase in the variability and magnitude of the precipitation events, leading to both flooding and droughts (Collins et al. 2013), will likely impact the $CH_4$ production and oxidation processes in the boreal peatlands.

Peatland succession typically entails a change in the methanogenic pathways from acetoclastic to hydrogenotrophic $CH_4$ production. Although this is mostly linked to the development from mesotrophic fens to ombrotrophic bogs and related change in the vegetation and the pH (Juottonen et al. 2005; Galand et al. 2005; Rooney-Varga et al. 2007; Kotsyurbenko et al. 2007, Hines et al. 2008), the same pattern has been evidenced in the thawing permafrost (Mondav et al. 2014; Liebner et al. 2015). The MOB communities of the northern acidic peatlands seem to be dominated by the alphaproteobacterial type II MOB, especially the *Methylocystis* species (e.g. Dedysh 2001; 2003; Yrjälä et al. 2011; Chen et al. 2008). However, the type I MOB seem to be abundant (Jaatinen et al. 2005) and active (Morris et al. 2002, Chen et al. 2008; Gupta et al. 2012; Peltoniemi et al. 2016) in the more mesotrophic, fen-type peatlands. In addition to the differences between the peatland types, both methanogen and MOB communities vary along smaller spatial scales and seasons, which may be a factor behind the high variability of $CH_4$ emissions (Bridgham et al. 2013).

### *1.4.2 Methane turnover in disturbed and restored boreal peatlands*

Due to the WT lowering induced by the drainage, aerobic peat decomposition accelerates and leads to an increase in the $CO_2$ emissions (e.g. Komulainen et al. 1999, Salm et al. 2012). Simultaneously, the $CH_4$ emissions decrease (e.g. Martikainen et al. 1995, Roulet and Moore 1995) but the system will still switch from a C sink to a source, if the C losses are not

compensated through for example afforestation (Ojanen et al. 2013, Maljanen et al. 2010). The peat extraction has an even more detrimental effect, as the living vegetation and the peat layers are removed. In addition to losing the C accumulated as peat, the abandoned peat extraction sites are generally carbon sources due to the aerobic degradation of the residual peat (Waddington et al. 2002; McNeil and Waddington 2003; Salm et al. 2012).

Peatland restoration aims to return the functions typical to a pristine ecosystem – including the $CH_4$ production in the peat layers. Firstly, the WT level needs to be returned through blocking of the drainage ditches and, in the case of the forested sites, possible removal of the tree-stand (Tarvainen et al. 2013). With submerged conditions, the peatland vegetation may return either spontaneously (Tuittila et al. 2000b; Maanavilja et al. 2014; Laine et al. 2016) or through artificial reintroduction through transplantations: Especially in Northern America, addition of *Sphagnum* diaspores on the extracted peat basin is a typical way to enhance the restoration process (Rochefort et al. 2003, Chimner et al. 2017). Based on $CH_4$ emission measurements, the $CH_4$ production may re-activate relatively rapidly after the initiation of a higher WT and re-vegetation, but the recovery rate depends for example on the peatland type and the degree of disturbance (Tuittila et al. 2000a, Waddington and Day 2007; Abdalla et al. 2016). Later, an increase in the *Sphagnum* moss cover seems to lead to decreased $CH_4$ emissions (Waddinton and Day 2007) – a pattern similar to the pristine peatland succession.

Compared to pristine peatlands, the methanogenic and methanotrophic communities of disturbed sites are not as well documented. While their activity [(potential $CH_4$ production/oxidation (MPP/MOP)] has been studied in both forestry-drained (e.g. Juottonen et al. 2012; Mastný et al., 2016) and cutover sites (Francez et al. 2000; Glatzel et al. 2004; Basiliko et al. 2007; Strack et al. 2017), their community structures have received less attention (Urbanová et al. 2011; Basiliko et al. 2013; Juottonen et al. 2012; Reumer et al. 2018).

### 1.5 *Sphagnum* - a key species of peatlands

Boreal peatlands would not exist without one plant genus, the *Sphagnum* mosses. These mosses characterize the peat ecosystems with a coverage typically reaching almost 100% in older, ombrotrophic bogs (Rydin and Jeglum 2013). Accordingly, the *Sphagnum* moss photosynthesis is responsible of most of the carbon accumulation and, due to its low degradability, *Sphagnum* detritus is estimated to provide 50% of the peat in the boreal peatlands (Turetsky 2003).

The reason behind the dominance of *Sphagnum* mosses is their ability to engineer their surroundings to favor their proliferation on the expense of other plants (Rydin and Jeglum 2013). One reason for their success, and the acidification development during the fen-bog transition, was long believed to be their high cation exchange capacity, which would lower the pH by changing protons for the positive cations present in the pore water (Clymo and Hayward 1982; van Breemen 1995). However, the *Sphagnum* dominance over for example vascular plants seems to be mainly caused by the thickening *Sphagnum* layer that disconnects the surface vegetation from the minerals provided by the ground water (Hughes and Barber 2004; Soudzilovskaia et al. 2010). In part, the slowing decomposition and the increasing peat accumulation are aided by the antimicrobial properties and the high recalcitrance of the *Sphagnum* mosses (van Breemen 1995; Hájek et al. 2011).

The genus *Sphagnum* belongs to the phylum Bryophyta, which is one of the oldest terrestrial plant lineages (Bateman et al. 1998). However, it is estimated that most of the current 300-500 *Sphagnum* species have originated fairly recently, < 20 million years ago, together with the emergence of the boreal peatland ecosystems (Shaw et al. 2010). Different *Sphagnum* species vary with their growth preferences with some of them typical on minerotrophic fens (e.g. *S. riparium*, *S. warnsdorfii*) and some on more acidic, ombrotrophic bogs (e.g. *S. fuscum*, *S. rubellum*, *S. cuspidatum*). *Sphagnum* species differ also in the hydrological adaptation, which leads to the formation of microtopography: Species able to conduct water to their growth form, such as the section Acutifolia, form dense hummocks above the WT, whereas species adapted to photosynthesis below the WT, such as section Cuspidata, can be found in the wet depressions, known as hollows or flarks (Johnson et al., 2015). Between these there are non-hummock forming species thriving in the flat, intermediate microforms known as the lawns. Formation of the microhabitats is an important feature that increases the functional biodiversity of the mosses and as such, the ecosystem resilience to disturbances such as wildfires (Benscoter and Wieder 2003) and even climate change (Korrensalo et al., 2017).

## 1.6 The *Sphagnum* microbiome

The microbiome of a plant can be considered to include only the endophytic organisms living inside the plant cells (in the endophere) or it may include also the organisms tightly bound to the plant surface (the ectosphere), which is the concept used in this thesis work. Either way, these microbes live in an association with the plant, which may give them advantages when compared to a life as a free-living organism. In turn, the endophytes can provide certain benefits to the host plant, such as nutrient acquisition or suppression of plant pathogens (Liu et al. 2017). Despite their life inside plant cells, pathogens are usually not considered endophytes (Hardoim et al. 2008). The endophytes can be either seed-borne i.e. may live their whole life cycle within the plant (obligate endophytes), or they may colonize the plant for example from the soil and spend only parts of their life inside the plant (facultative endophytes). The latter group can be either opportunists that take occasional advantage of the benefits offered by the plant (e.g. nutrients and protection) or they may be just passively floating within the plant without receiving any positive gains (Hardoim et al. 2008).

Within *Sphagnum*, the endophytes most often live inside the dead, water-filled hyaline cells (Raghoebarsing et al. 2005; Bragina et al. 2012a) that enable the high water-holding capacity of these mosses and are found throughout the moss leaves and stem. The pores of the hyaline cells allow the movement of water as well as small particles, including bacteria, through their walls (Rydin and Clymo 1989). In addition to more species/function-specific benefits, the hyaline cells are believed to offer a more stable, less acidic habitat within the harsh peatland environment (Kostka et al. 2016). Based on a recent metagenomics survey, the *Sphagnum* microbiome is well adapted to the oxidative stress entailed in the flooding-desiccation cycles typical for the moss environment (Bragina et al. 2014).

### *1.6.1 Sphagnum-associated methanotrophs*

Methane oxidation has been long known to occur in the peat layers, but the concept of living *Sphagnum* mosses as a habitat for the MOB is relatively new (Table 2). Vasil'eva et al. (1999)

Table 2. Studies targeting *Sphagnum*-associated methanotrophs (SAM) and/or their activity (in a chronological order based on the publication year). For the abbreviations, see page 8. Table continues on the next page.

| Reference | Main methods | *Sphagnum* species | Type of MOB detected | Main results (related to SAM) |
|---|---|---|---|---|
| Vasil'eva *et al*. (1999) | Cultivation (enrichements), microscopy, MOP | not mentioned | Morphologically similar to e.g. *Methylobacter* | Acidophilic MOB-type cells were present in the *Sphagnum* hyaline cells. *Sphagnum*-MOB association was found to be active in several locations over the Russian permafrost area. |
| Frenzel and Karofeld, (2000) | $CH_4$ flux, pore water $CH_4$ concentration, MOP and MPP of the peat layer | *S. fuscum*, *S. rubellum, S. cuspidatum*, *S. balticum* | na | In the hollows, $CH_4$ oxidation occurred in the lower green *Sphagnum* parts. |
| Basiliko et al. (2004) | MOP, pore water $CH_4$ concentration | *S. magellanicum*, *S. capillifolium, S. majus* | na | Different *Sphagnum* moss species had a variable ability to oxidize $CH_4$, although inter-species differences were small compared to differences across habitats. |
| Raghoebarsing et al. (2005) | 16S rDNA cloning/sequencing, FISH, MOP, electron microscopy, $^{13}CH_4$ labeling and lipid analysis, isotopic mass balancing | *S. cuspidatum*, *S. magellanicum*, *S. papillosum* | 93 % similarity to *Methylocella*/*Methylocapsa* | MOB have a symbiotic relationship with *Sphagnum*. MOB related bacteria detected in the hyaline cells. |
| Berestovskaya et al., (2005) | $CH_4$ flux, MPP, MOB cultivation and MOP in 5 °C and 15 °C | *Sphagnum* sp., *S. fuscum,* | *Methylocella*, type I MOB | $CH_4$ oxidation was detected in green *Sphagnum* parts and in the bog water, but not in other tested mosses/lichens. |
| Larmola et al. (2010) | MOP, transplantation, stable carbon isotope ratio analysis, DGGE, pore water $CH_4$ concentration | *23 species* | *Methylocystis* | All 23 species showed methanotrophy, transplantation to $CH_4$ oxidation positive environment induces the activity in inactive mosses. |
| Kip et al. (2010) | MOP, $CH_4$ flux, isotopic labeling of lipids and chlorophyll with $^{13}CH_4$, *pmoA* microarray, *mmoX* PCR | *S. cuspidatum*, *S. magellanicum* | high diversity (type I and II) | SAM were found globally with high diversity, $CH_4$ oxidation increased with temperature and was highest in the pools, $CH_4$-derived carbon was incorporated to the mosses. |
| van Winden et al. (2010) | Lipid biomarker analysis after SIP with $^{13}CH_4$ | *S. cuspidatum*, *S. magellanicum* | - | No unambiguous biomarker for SAM could be found. |

| Reference | Main methods | *Sphagnum* species | Type of MOB detected | Main results (related to SAM) |
|---|---|---|---|---|
| Kip et al. (2011a) | $CH_4$ flux, pore water $CH_4$ concentration, MPP, MOP, *pmoA* microarray, cloning (*pmoA* +16S rRNA), *mmoX* + Verrucomicrobia PCR | *S. magellanicum* | high diversity of type I and II | SAM activity and $CH_4$ concentration correlated, activity was highest in the pools, MOB diversity in the Patagonian peatland similar in the different microhabitats and similar to northern hemisphere peatlands, similar communities had differing activity. |
| Kip et al. (2011b) | Cultivation, *pmoA* microarray, identification tests | *S. cuspidatum*, *S. denticulatum* | high diversity of type I and II | High diversity of SAM isolates gained, first acidophilic/-tolerant gammaproteobacterial SAM isolated. |
| Kip et al. (2011c) | *pmoA* microarray, 454 high-throughput sequencing, *mmoX* cloning | not mentioned | *Methylomonas*, *Methylocystis*, other type I and II | Microarray and pyrosequencing revealed similar SAM diversity. |
| Parmentier et al. (2011) | $CH_4$ flux, MOP, modelling | *Sphagnum* spp. | na | Compared to the other vegetation types, $CH_4$ emissions were 50% lower when *Sphagnum* was the dominant plant type |
| van Winden et al. (2012) | Analysis of bacteriohopanepolyols from MOB strains | *S. cuspidatum* | na | Type I and II SAM strains differ in their bacteriohopanepolyol profile. |
| Bragina et al. (2013a) | qPCR, high-throughput sequencing + network analysis (*nifH, pmoA*) | *S. magellanicum*, *S. fallax* | mainly *Methylomonas*, *Methylocystis* | Microbial group's specificity to *Sphagnum* species depends on its function. |
| Larmola et al. (2014) | $^{15}N_2$ + $^{13}CH_4$-labeling experiment in the field, analysis of $\delta^{15}N$ and $\delta^{13}C$ values and C and N contents | Several species dominant on the sites | na | Methanotrophy induced $N_2$ fixation provides over one third of the nitrogen input in the young peatland succession stages. |
| Vile et al. (2014) | $N_2$-fixation measurement with the acetylene reduction assay, cloning and qPCR of the *nifH* and 16S rDNA genes and transcripts | *S. fuscum*, *S. angustifolium*, *S. capillifolium* | type II | MOB, not cyanobacteria, were responsible for the $N_2$ fixation in *Sphagnum.* |
| Leppänen et al. (2015) | $^{15}N_2$+$^{13}CH_4$-labeling experiment in the laboratory, DGGE and cloning (*nifH)* | *S. fimbriatum*, *S. balticum*, *S. fallax, S. riparium* | Diatzotrophs from the order *Rhizobiales* (not close to cultured MOB) | *Sphagnum*-associated $N_2$ fixation was controlled by the moss species and hydrology. MOB were not responsible for the $N_2$ fixation in the studied fen and forest mosses. |
| Reumer et al. (2018) | MOP (for peat: MPP, qPCR, Miseq sequencing of the *mcrA*, T-RFLP of the *pmoA*) | *S. fimbriatum*, *S. flexuosum*, *S. fallax*, *S. capillifolium* | na | *Sphagnum*-associated MOP did not differ significantly between the restored and pristine bog sites. |

were among the first to suggest that the MOB live in the dead hyaline cells inside the living plant. Results from Frentzel and Karofeld (2000) supported this finding as they linked the highest $CH_4$ oxidation to the lowest green parts of the *Sphagnum* mosses (in hollows). Basiliko et al. (2004) further investigated this phenomenon and, again, located the highest oxidation potential in the lower, non-photosyntetic parts of the mosses. Soon after, the concept of *Sphagnum*-associated methanotrophs (SAM) was confirmed by molecular detection of MOB-type bacteria inside the hyaline cells (Raghoebarsing et al. 2005). The detected MOB were relatively distant to the known MOB strains (93% 16S rRNA similarity to *Methylocella*/*Methylocapsa*), but similar sequences were later discovered in a West Siberian *Sphagnum* bog (Dedysh et al., 2006). The metabolic route of the $CH_4$-derived C was traced from the SAM to the *Sphagnum* using a $^{13}CH_4$ labeling analysis of bishomohopanoic acid and phytosterols/chlorophyll *a* (Raghoebarsing et al. 2005; Kip et al. 2010), demonstrating the mechanism of carbon recycling in the bog ecosystem. The incorporation of $CH_4$-C to the mosses seems to be highest in the submerged conditions, highlighting the usefulness of this $CO_2$ source when otherwise depending on the slow diffusion of the $CO_2$ from the water phase (Larmola et al. 2010a; Kip et al. 2010). An estimated incorporation of 5% to 30% of the $CH_4$-C to the mosses would help to explain the high level of C accumulation despite the slow primary production in the peatland ecosystems (Raghoebarsing et al. 2005; Larmola et al. 2010a). Liebner et al. (2011) recorded a similar connection between the MOB and brown mosses (*Scorpidium scorpioides*) in Siberian tundra ponds and, based on the higher $CH_4$ oxidation during the photosynthesis than in the dark, demonstrated the benefit of plant-derived oxygen for the SAM. The importance of the *S. scorpioides*-associated $CH_4$ oxidation has been shown also by Knoblauch et al. (2015).

Studies conducted/published simultaneously with this thesis work have revealed the high prevalence of the SAM both geographically (Kip et al. 2010) and within different *Sphagnum* species (Larmola et al. 2010a). In a study of Patagonian *S. magellanicum* mosses, the SAM community composition did not vary between different microhabitats (Kip et al., 2011a) but in another study, those differences were still larger than the difference between the *Sphagnum* species (Basiliko et al. 2004). Only few SAM strains have been isolated (Kip et al. 2011b) and most of the studies rely on molecular methods in the SAM community analysis. Overall, the understanding of the SAM ecology is quite poor and very little is known for example of the effects of different environmental changes, such as peatland succession, on the SAM communities or their activity. Although the SAM seem to clearly benefit from a close association to the mosses, it is still unclear how tightly these organisms are connected – a feature that might have implications on their response to abiotic factors.

In addition to the $CH_4$ oxidation, SAM are major contributors to the peatland nitrogen cycle. Especially in the bogs, which are disconnected from the minerotrophic ground water flow, the atmospheric N deposition is complemented with diatzotrophic $N_2$ fixation. Due to the use of acetylene reduction assay that targets mainly cyanobacteria (Hardy et al., 1968), methanotrophic $N_2$ fixation was long excluded as a source of nitrogen to peatlands. However, recent studies have shown that $CH_4$ oxidation induces $N_2$ fixation in the main primary producers in peatlands, i.e. the *Sphagnum* mosses (Larmola et al. 2014; Vile et al. 2014).

### *1.6.2 Non-methanotrophic microbes associated with Sphagnum*

Besides MOB, *Sphagnum* mosses harbor a high diversity of other closely associated microorganisms (Opelt and Berg 2004; Opelt et al. 2007a) that differs from the microbiome of other higher plants and peat soils (Bragina et al. 2014; 2015; Table 3). Unlike MOB, many

of these moss endophytes are specific to certain *Sphagnum* species and can spend their whole lifecycle within the mosses (Opelt et al. 2007b; Bragina et al. 2012a; 2012b; 2013b). The reason behind their species specificity, or the lack of it, seems to be the value of the specific microbial groups and their functions for the survival of the mosses. For example, the $N_2$ fixation is proposed to be essential for the plant primary production in the harsh ombrotrophic bog environment. Accordingly, in contrast to the *pmoA* gene, $N_2$ fixation specific *nifH* gene composition showed specificity for the *Sphagnum* species (Bragina et al. 2013a). However, despite this pattern, *Sphagnum* and the $N_2$ fixing diatzotrophs may not share a direct mutualistic symbiosis, as they seem to react differently to abiotic factors (van den Elzen et al. 2017). The general *Sphagnum* microbiome appears to be sensitive to climate change induced warming, which may lead to increased decomposition (Jassey et al. 2011; 2013).

While most studies have concentrated on the role of *Sphagnum*-associated microbes in the plant as well as in the functioning of the ecosystem, few studies have highlighted the biotechnological potential of the *Sphagnum* microbiome, such as the antagonists against fungal pathogens and their agricultural applications (Opelt and Berg 2004; Shcherbakov et al. 2013).

Table 3. *Sphagnum* microbiome studies targeting other microbial groups/functions (does not include studies exploring the upper, *Sphagnum* dominated peat bulk layer). For the abbreviations, see page 8. Table continues on the next page.

| Reference | Main methods | *Sphagnum* species | Targeted / detected microbial group / function | Main results |
|---|---|---|---|---|
| Opelt and Berg (2004) | SSCP, DGGE, cultivation | *S. rubellum* | General bacterial community | *Sphagnum* harbors bacteria antagonistic towards fungi that have the potential to be used in the biological control of fungal pathogens. |
| Opelt et al. (2007a) | Cultivation | *S. magellanicum*, *S. fallax* | General bacterial community | *Sphagnum* harbors high diversity of antagonistic bacteria towards fungi and also many human pathogens. |
| Opelt et al. (2007b) | Electron microscopy, SSCP, cloning, vegetation analysis | *S. magellanicum*, *S. fallax* | General bacterial community | High degree of host specificity between bacteria and *Sphagnum* species was found independent of the geographic location. |
| Jassey et al. (2011) | Microcosm experiment with a temperature gradient, analysis of phenolic compounds, microbial community analysis by microscopy | *S. fallax* | Primary producers, fungi, and unicellular predators, bacteria | Microbial communities of *Sphagnum* upper segments, and their relation to abiotic factors, changed with temperature. Phenolic compounds affected microbial composition. |
| Bragina et al. (2012a) | 16S rDNA SSCP + cloning, FISH, CLSM, 454 high-throughput sequencing of alphaproteobacteria | *S. magellanicum*, *S. fallax* | General bacterial community | Mosses maintain their specific bacterial diversity within the whole lifecycle, nutrients and pH modulated bacterial communities. |
| Bragina et al. (2012b) | Analysis of *Sphagnum* secondary metabolites with HPLC-PDA (high-performance liquid chromatography photodiode array), SSCP, FISH, CLSM, 454 high-throughput sequencing (*nifH*, 16S rDNA) | *S. fallax*, *S. angustifolium* | General bacterial community, diazotrophs | Similar type *Sphagnum* species have similar bacterial community patterns. |
| Berg et al. (2013) | Incubation, acetylene reduction assay | *S. riparium* | $N_2$ fixation, diatzotrophs | 35% of the N fixed by cyanobacteria was used by the S*phagnum* mosses. |

| Reference | Main methods | *Sphagnum* species | Targeted / detected microbial group / function | Main results |
|---|---|---|---|---|
| Jassey et al. (2013) | Warming experiment in the field, morphological analysis of different microbial group abundance/biomass, flow cytometry for bacterial counts, modelling | *S. fallax* | bacteria, fungi, microalgae, ciliates, testate amoebae, rotifers, and nematodes | Warming affected microbial food webs associated with *Sphagnum* (loss of predatory microbes, increase in bacteria) in a way that could lead to increased decomposition and thus to a decrease in the carbon storage. |
| Shcherbakov et al. (2013) | Cultivation | *S. magellanicum*, *S. fallax* | *Burkholderia*, *Pseudomonas*, *Flavobacterium*, *Serratia and Collimonas* | *Sphagnum*-associated endophytic bacteria could be utilized in agriculture to suppress fungal pathogens. |
| Bragina et al. (2013b) | 454 seqeuncing of the 16S rRNA , FISH | *S. magellanicum*, *S. fallax* | *Burkholderia* | *Burkholderia* transmit vertically between the *Sphagnum* mosses and are thus part of their core microbiome. |
| Bragina et al. (2014) | HiSeq sequencing, FISH, CLSM | *S. magellanicum* | Microbial metagenome associated with *Sphagnum* | *Sphagnum* has a unique and diverse microbiome, that enables its survival in changing environmental conditions |
| Bragina and Berg (2015) | MiSeq sequencing of the 16S rDNA | *S. magellanicum*, *S. angustifolium* | General bacterial community | Bog vegetation (plants and lichens) share a core microbiome. |

# 2 AIMS OF THE THESIS

Peatlands are a significant source of the greenhouse gas $CH_4$. *Sphagnum* mosses fix C not only from atmospheric $CO_2$ but also from the underlying peat layers through the activity of $CH_4$ oxidizing methanotrophic bacteria living inside the moss hyaline cells and on the moss surfaces. This phenomenon is of high importance for the mitigation of $CH_4$ emissions and C accumulation but the ecology of *Sphagnum*-associated methanotrophs (SAM) is still poorly understood - especially when compared to the MOB living in the bulk peat layers. This thesis aimed to characterize the response of SAM to environmental change. SAM dynamics were studied in connection to a direct change of the microhabitat (Study **I**) and in various ecosystem succession stages on both pristine (**II**) and disturbed peatlands (**III**). The following questions were addressed:

a) Is the symbiosis with the *Sphagnum* mosses obligatory or facultative for the SAM? Implications for resilience towards disturbance? (**I**, **II**, **III**)

b) Which community members are most actively oxidizing $CH_4$? How diverse are the SAM communities in general? (**II**)

c) How does the ecosystem level succession affect the SAM in pristine versus disturbed peatlands? Do the SAM differ between fens and bogs? (**II**, **III**)

d) How does SAM community composition and activity relate to the $CH_4$ turnover in the peat layers? (**III**)

# 3 MATERIALS AND METHODS

## 3.1 Study designs and locations

The dynamics of the SAM communities and their activity were analyzed in three different studies that included a pristine ombrotrophic bog and an oligotrophic fen (**I**), a chronosequence of pristine sites from young meadows and fens to the bog stage (**II**) and a re-vegetation gradient of fen-type cutover sites (**III**; Table 4).

**I. Transplantation and bathing experiments to reveal the movement of SAM between the mosses (an indication of a facultative association)**
This study consisted of two separate experiments. First a transplantation study was conducted in the Lakkasuo raised bog complex, Finland 61˚48'N, 24˚19'E). The aim was to test the movement of MOB between the moss plants in field conditions – a trait that was considered to imply a facultative connection to the mosses. Samples of *Sphagnum rubellum* ("immigrants"), that showed no detectable $CH_4$ oxidation in the laboratory incubations, were planted in six different flark sites harboring *Sphagnum* with known high $CH_4$ oxidation activity (Figs. 1 and 2). As a control, the same *S. rubellum* plants were also re-planted in their original site and the Sphagna from the active sites ("natives") were gathered and re-planted in their original sites. All these transplanted mosses were sampled on days 0, 3 and 28 from the start of the experiment. After washing and drying to remove the loosely attached microbes etc., the 10 uppermost cm of the mosses were used in the further analysis aiming to reveal the effect of transplantations to the activity and community composition of the SAM. Possible increased similarity between the communities of immigrant and native mosses was considered a sign of potential water-mediated movement.

Table 4. Locations and types of the pristine (**I, II**) and disturbed sites (**III**) sampled in this thesis.

| Study | Location | Coordinates | Peatland type | pH |
|---|---|---|---|---|
| I | Lakkasuo | 61˚48'N, 24˚19'E | Ombrotrophic bog | 4.1[a] |
| | Sallie's fen | 43˚13'N, 71˚04'W | Oligotrophic fen | 4.5[b] |
| II | Siikajoki | 64°45'N, 24°42'E | Gradient from young wet meadows to bogs | 4.5–6.5 |
| III | Aitoneva | 62°12′N, 23°18′E | Re-vegetation gradient of cutover fens | 4–5.5 |

[a] Average pore water value according to Laine et al. (2004)

[b] Average value for sites with *S. magellanicum* according to Carroll et al. (1997)

To get further evidence for the actual movement, and not just activation of the original SAM community, a second experiment was conducted with *Sphagnum* mosses and active flark water gathered from Sallie's Fen in NH, USA (43˚12.5'N, 71˚03.5'W). The aim was to clarify, if just the water from a flark with active $CH_4$ oxidation was enough to transmit methanotrophs and $CH_4$ oxidation activity to the inactive mosses. For this purpose, five treatments were conducted: 1) fresh inactive *S. magellanicum* samples were bathed overnight (11h) in untreated active flark water and 2) in the same water after 0.45 um filtration to remove the methanotrophs. After the bathing in active water, a partial sample of the mosses was rinsed with deionized water (3). As controls, also the original, unbathed *S. magellanicum* samples and *S. majus* from the active flark site were included in the following analysis. After these treatments, MOP and type II *pmoA* abundance was analyzed from the samples.

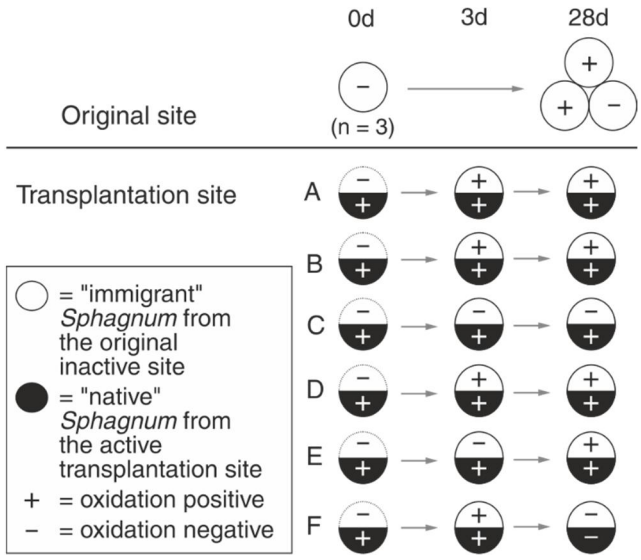


Figure 1. Design of the transplantation experiment and the presence of detectable $CH_4$ oxidation potential (>0.005 µmol $CH_4$ $g^{-1}$ $h^{-1}$) in the moss samples during the experiment. Adopted from the paper **I**.

Figure 2. Transplanted *Sphagnum* mosses in the Lakkasuo bog in the study **I** (photo by Tuula Larmola).

**II. Analysis of active SAM diversity on a pristine succession gradient**

This study was conducted on the land uplift coast of Bothia in the Western Finland (Siikajoki, 64°45'N, 24°42'E). As the land has slowly emerged from the sea, a natural chronoseqeunce of peatland stages from young wet meadows to old ombrotrophic bogs has been formed on this area (Fig. 3 in this summary; Table 1 in **II**). It has been a target of several other studies as well (e.g. Merilä et al. 2006; Tuittila et al. 2013; Laine et al. 2016). The aim was to collect several *Sphagnum* samples from various successional stages for the analysis of which type of MOB are active in the mosses in general and to see the effect of successional stage and *Sphagnum* species on the activity and diversity of the SAM. The sampling included 41 *Sphagnum* samples representing the dominant species of each site. From these samples, 12 samples showing active $CH_4$ oxidation were chosen to the molecular community analysis.

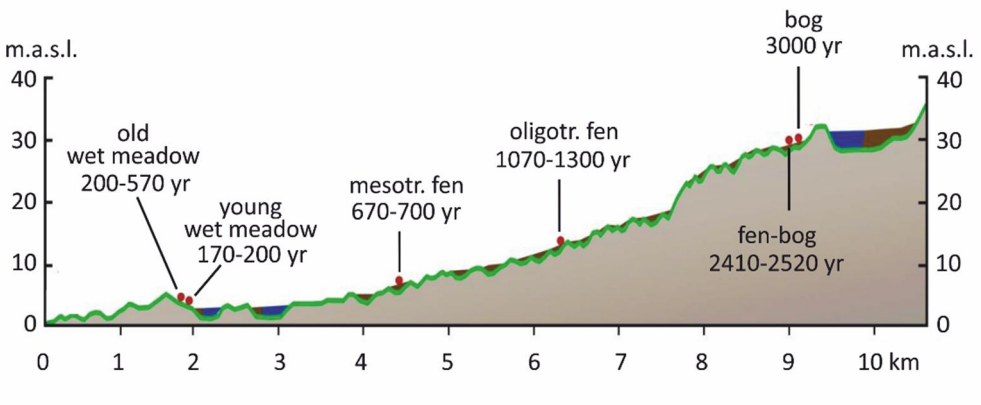


Figure 3. Profile of the pristine succession gradient on the land up-lift coast of the Bothnia Gulf, sampled in the study **II**. Figure shows the six stages from the young meadow to the old ombrotrophic bog together with their ages, estimated based on the land up-lift rate or radiocarbon dating (m.a.s.l. = meters above sea level, mesotr. = mesotrophic, oligotr. = oligotrophic). Modified from a figure originally drawn by Irene Murtovaara and used in Merilä et al. (2006).

Figure 4. Pictures from the re-vegetating cutover gradient (study **III**) showing the sites restored 2 years (AN2y, on the left), 17 years (AN17y) and 63 years (AN63y) before the sampling, together with the pristine reference site (PRST, on the right).

**III. Analysis of $CH_4$ turnover during the re-vegetation succession of cutover peatlands**

This study was conducted on the oldest peat extraction area of Finland, Aitoneva, in southern Finland (62°12′N, 23°18′E). The aim was to see the effect of re-vegetation process on the $CH_4$ cycling microbes, thus also enabling the comparison of the SAM to the succession of the MOB and the methanogens living in the peat layer. Both the *Sphagnum* and the peat (layers 10-20 cm and 20-30 cm from the moss surface) were sampled in triplicate on three cutover sites representing different re-vegetation stages: 2 years, 17 years and 63 years from the rewetting/restoration (AN2y, AN17y and AN63y; Fig. 4). A nearby pristine fen (PRST) representing the target stage of successful re-vegetation was sampled as a reference, including also the bottom peat layer that was considered to represent the same, highly decomposed layer that formed the surface of the recently abandoned cutover site, AN2y. On the youngest site, extracted using the milling technique, restoration included also spreading of *Sphagnum* diaspores to aid the re-vegetation. On the 17 years old site, also extracted with milling, the ditches had been manually blocked to rewet the site but no other restoration measures had been conducted. Due to the use of the older block-cut extraction method, the oldest site (AN63y) had started to recover naturally soon after the end of the peat extraction. In connection with the sampling, the vegetation was characterized on each of the sampling spots to link the microbial markers to the three main plant functional types: Sphagna, sedges and shrubs.

## 3.2 Analysis methods

In all three studies, the analyses of the SAM included only the topmost 10 cm of the living *Sphagnum* mosses (green parts). Before the analyses, the moss samples were thoroughly washed with deionized water to remove the loosely associated microbes from the moss surface. The bulk peat layers were sampled separately for the analysis of peat inhabiting MOB (**III**).

Due to reasons such as syntrophic associations and other complex interactions among environmental microbes, cultivation of these organisms is often highly challenging. Consequently, the detection and analysis of organisms such as MOB typically relies on molecular methods based on the nucleic acids (DNA, RNA). In this thesis, the MOB were analyzed mainly with a *pmoA* gene based microarray, which was composed of over 130 probes aimed to cover the currently known *pmoA* diversity (Bodrossy et al. 2003; Stralis-Pavese et al. 2004; 2011). In addition, in the second study (**II**), the 16S rRNA gene diversity was explored from the $^{13}$C-labeled DNA originating from the stable isotope probing (SIP) of the moss samples. The DNA-SIP is based on a sample incubation with a labeled substrate, here $^{13}C$-$CH_4$, and the subsequent ultracentrifugation to separate the labeled DNA of the actively metabolizing community from the unlabeled background DNA (Radajewski et al. 2003). In the last study (**III**), the microarray was complemented with a qPCR analysis specific to the three MOB types Ia, Ib and II, and a separate PCR analysis of the *mmoX* gene. All three studies included the measurement of the MOP using a laboratory incubation setup that was otherwise similar (the same equipment, 120 ml flasks, incubation in + 15 °C) except for the $CH_4$ concentration, which was either 10 000 ppm (**I**, **II**) or 1000 ppm (**III**). The reason for this difference was the aim to compare the results of **I** and **II** to the results of Larmola et al. (2010a), whereas in **III**, the MOP were primarily compared between the moss and the peat layers and the layout was kept similar to the previous studies focusing on the peat layer (e.g. Kotiaho et al. 2008; 201x). Both concentrations were considered to be non-limiting for the low-affinity MOB typical for the $CH_4$ producing environments such as peatlands.

The methods used in the different studies are listed in Table 5. Their details can be found in the papers **I**, **II** and **III**. In addition, the following environmental variables were measured: the WT level at the time of sampling (**I**, **II**, **III**), the $CH_4$ concentration of the pore water (**I**, **II**), the temperature of the peat (**I**), the pH of the peat (**II**, **III**), the peat bulk density (**III**) and the vegetation composition (**III**).

Table 5. Methods used in the studies **I**, **II** and **III**. For the abbreviations, see page 8.

| Method | Aim | Described and used in |
|---|---|---|
| Incubation methods | | |
| Potential $CH_4$ oxidation | Measuring the potential activity of methanotrophs | I, II, III |
| Potential $CH_4$ production | Measuring the potential activity of methanogens | III |
| Stable isotope probing with $^{13}CH_4$ | Labeling the methanotrophs actively oxidizing $CH_4$ | II |
| Molecular methods | | |
| DNA extraction from *Sphagnum*/peat | - | I, II, III |
| qPCR amplification of the *pmoA* gene | Quantification of the methanotrophs with pMMO | I, III |
| qPCR amplification of the *mcrA* gene | Quantification of the methanogens | III |
| *pmoA* gene microarray | Characterization of the methanotrophic communities | I, II, III |
| DGGE and Sanger sequencing of the *pmoA* gene | Characterization of the methanotrophic communities | I |
| T-RFLP of the *mcrA* gene | Characterization of the methanogenic community structure | III |
| Cloning, RFLP and Sanger sequencing of the 16S rRNA gene | Characterization of the total bacterial community associated with active $CH_4$ oxidation | II |
| PCR of the *mmox* gene | Detection of the methanotrophs with sMMO | III |
| Statistical methods | | |
| Multivariate methods (RDA, pRDA, CCA, PCA, NMDS, variation partitioning and/or ANOSIM) | Analysis of the relationships between community structures in different samples and impact of environmental variables on the communities | I, II, III |
| ANOVA - Tukey's HSD and/or Kruska-wallis – Nemenyi | Test the statistically significant differences of single variables between samples | I, II, III |
| Correlation analysis (Spearman correlations ($r_s$), mixed effects model and/or linear regression | Analysis of the relationships between different variables | II, III |
| Diversity and evenness indices | Comparison of the community structures | II |
| Phylogenetic analysis of 16S rDNA sequences | Characterization of the bacterial community members | II |

# 4 MAIN RESULTS

## 4.1 Water as a route for SAM dispersal between the mosses

The first study (**I**) continued the work presented in Larmola et al. (2010a), which concluded that inactive mosses become active in $CH_4$ oxidation when moved next to active mosses. The thesis work aimed to reveal whether the SAM were actually moving between the plants in the water phase or whether the suitable conditions just activated the SAM population already present in the inactive mosses. This was tested both in the field and in the laboratory in two different experiments.

In the transplantation experiment, four out of six inactive mosses gained detectable MOP already within 3 days after being planted next to *Sphagnum* mosses with known high MOP (Fig. 1). Similarly, on the day 28, four originally inactive mosses showed detectable MOP with only one out of six showing no MOP on either day 3 or 28. On day 28, the MOP rate did not differ between the originally inactive and the "native" mosses (Fig. 5). A community composition analysis with *pmoA* microarray showed that, in most of these transplanted

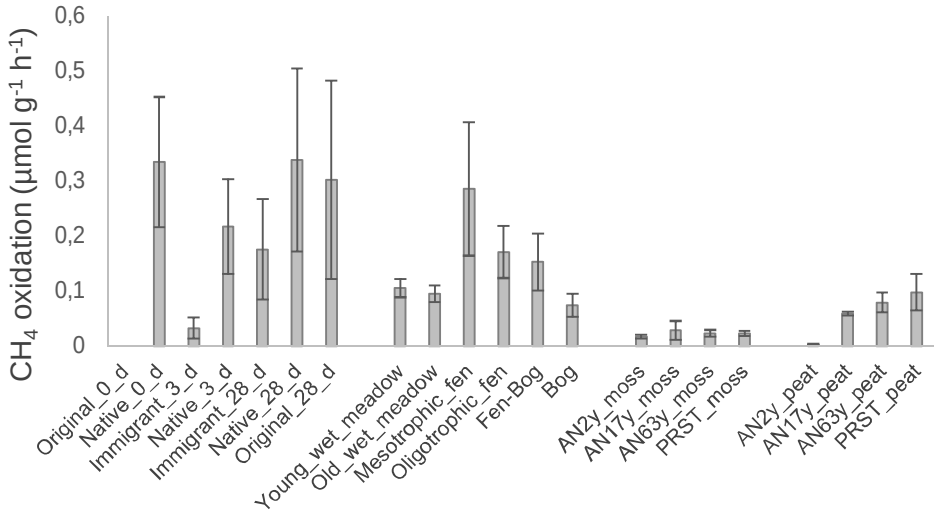


Figure 5. Potential $CH_4$ oxidation of the *Sphagnum* samples of the transplantation experiment (**I**), of the pristine succession gradient (**II**) and of the re-vegetation gradient of the cutover sites (**III**), together with the upper peat (-15 cm layer) samples from the cutover sites (**III**). The "original" mosses did not show any detectable $CH_4$ oxidation on day 0 (**I**). Within each study, none of the presented samples that showed detectable MOP differed significantly from each other, except for the AN2y_peat from the other peat samples. In the studies **I** and **II**, the measurements differed from the study **III** in terms of the initial $CH_4$ concentration (10 000 ppm vs. 1000 ppm) and the sampling time (June vs September). The results of the bathing experiment (**I**) are presented in Fig. 7 [original = samples of the original site, immigrant = originally inactive samples transplanted in the active flark site, native = mosses native to the active flark; AN = Aitoneva peat extraction area, PRST = pristine reference site, n=3/6 (**I**); n=3–8 (**II**) or n=3 (**III**)].

mosses, the SAM community of the "immigrants" started to become more similar with the "native" (originally active) mosses already after 3 days and after 28 days, the "immigrants" resembled the "native" SAM more than the original communities on day 0 (Fig. 6 in this summary, Fig. A1 in **I**). This change in the SAM composition, as demonstrated by a PCA analysis of both the individual samples and by the combination of all six samples, indicated the movement of SAM between the mosses. However, the link between the detectable MOP and community change was not entirely straightforward: on one of the samples, the MOP was activated even without major changes in the SAM composition – indicating activation of the original community when transplanted to the more favorable conditions. On the other hand, also the control inactive mosses planted in their original location gained activity (two out of three) and showed changes in their SAM community after 28 days - demonstrating both the temporal changes within location and possible movement of the new SAM from the surrounding environment.

The type II MOB were present in all tested samples with high diversity [Table 6 in this summary (pages 34–35), Fig. 3 in **I**] and thus the movement of this group was not clearly detectable. In contrast, the type I MOB showed clearer signs of movement between the mosses. They were not detected in any of the original inactive mosses (Table 6) but appeared in five of the six samples after the transplantation. The type Ib were detected also in the original inactive site on days 28 but the type Ia MOB were present only in the original active flark site. However, there was no clear link between the movement of certain MOB types and the activation of $CH_4$ oxidation.

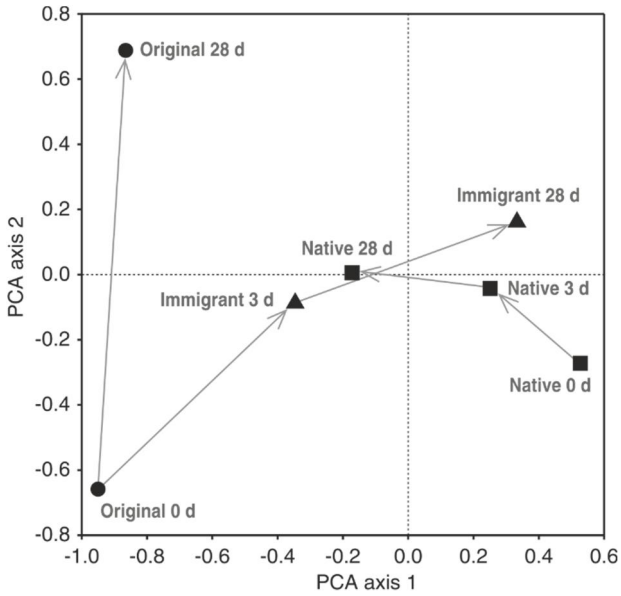


Figure 6. Development of the SAM community compositions (sample means) in the transplanted mosses during the 28 day long experiment (**I**) based on the principal component analysis (PCA) of the *pmoA* microarray data [original = samples of the original inactive site (n=1/3), immigrant = originally inactive samples transplanted in the active flark site (n=6), native = mosses native to the active flark (n=6)]. The PCA axes 1 and 2 explain 25% and 11% of the variation, respectively. Adopted from the paper **I**.

The possible SAM movement between the mosses was further tested in the second experiment. This time similar inactive mosses as in the transplantation experiment were bathed in pore water gathered from a flark showing active $CH_4$ oxidation. Results showed fast MOB colonization of the mosses during an overnight bathing, as demonstrated by a two-order rise in the type II MOB *pmoA* gene copy numbers and the initiation of the MOP when compared to an unbathed control and to a sample bathed in filter-sterilized flark water (Fig. 7). The detected SAM were indeed tightly associated with the Sphagna as a water rinse after the bathing did not have a significant effect on the results. The results were further confirmed by a DGGE analysis, where two *Methylocystis* bands were strongly visible in the samples bathed in the active water and, in contrast, absent when filtered water was used in the bathing (Fig 5. in **I**).

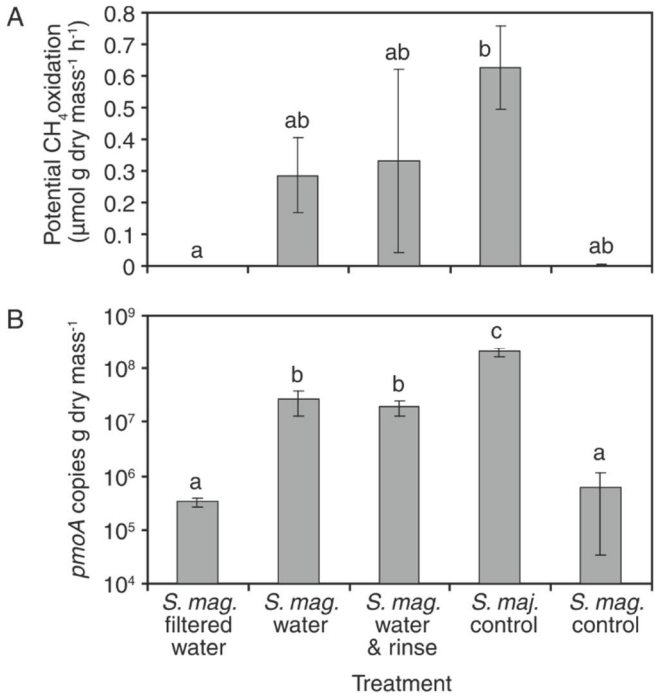


Figure 7. Potential $CH_4$ oxidation (A) and the abundances of the type II MOB *pmoA* genes (B) in the *Sphagnum* samples from the different bathing treatments (**I**). Same letter superscripts designate non-significant differences between treatments in the potential oxidation rates (S. mag .= *Sphagnum magellanicum*, S. maj. = *Sphagnum majus*, n=3, mean ± standard error). Adopted from the paper **I**.

## 4.2 SAM response to ecosystem succession

### *4.2.1 SAM on a pristine succession gradient*

The community composition of the active and total SAM communities was studied on a pristine peatland succession gradient formed due to a still ongoing land uplifting on the coast of the Bothnia Gulf (Table 1. in **II**). This setting allowed the examination of the general diversity of the SAM within different *Sphagnum* species and, concurrently, a study on the effect of peatland succession that entails a change in various environmental variables. To reveal the active SAM communities, SIP with $^{13}CH_4$ was used in a laboratory setup.

Based on the *pmoA* microarray analysis, the whole gradient from the youngest wet meadows to the fen-bog transition stage harbored a high SAM diversity (Table 6 in this summary, Figs. 1 and 3 in **II**). Out of the three separate fractions analyzed (un-incubated, $^{12}C$- and $^{13}C$-labeled), the SAM of the original un-incubated mosses had the highest diversity (Shannon index). However, only the diversity of active $^{13}C$-labeled SAM differed between the succession stages with an increase towards the older stages and a significant diversity difference between the young (1-3) and the old (4-5) stages. Samples from the bog (stage 6) did not consume enough $^{13}C$-$CH_4$ to be included in the molecular analysis.

The community compositions differed between the young (1-3) and the older (4-5) stages in all three DNA fractions [PCA (Fig. 2 in **II**), ANOSIM]. In the un-incubated mosses, the type Ia MOB were more prevalent in the young stages and type Ib in the old. The type II MOB, mainly *Methylocystis*, was more diverse in the $^{12}C$-DNA-fraction than in $^{13}C$ and seemed to be active only in the older stages. Based on the $^{13}C$-fraction, active type Ia MOB, especially *Methylomonas*, were relatively abundant and diverse throughout the succession stages and dominated especially in the youngest stages. In contrast, the active type Ib abundance and diversity increased towards the older stages, with freshwater group LW21 being most abundant.

The active SAM diversity was further characterized through cloning and sequencing of the universal 16S rRNA genes from the $^{13}C$-labeled DNA fraction. Here, the aim was mainly qualitative: to discover possible new SAM not covered by the *pmoA* microarray probes and to trace non-methanotrophic bacteria assimilating the $^{13}CH_4$ oxidation-derived $CO_2$ and thus closely connected to SAM. The sequence data concurred well with the microarray as 34% of the sequences belonged to type Ia, 7.5% to type Ib and 14% to type II MOB. In addition to the MOB species covered by the microarray, the putative *Methylocella* and *Methyloferula*, that lack the *pmoA* gene, were revealed as members of the presumably active SAM community (Figs. 4 and 5 in **II**). Among all the sequenced clones, 45% could not be affiliated to any of the known MOB. Within these bacteria, the presumably non-methanotrophic *Verrucomicrobia* were the largest group (15% of all clones sequenced) (Figs. 4 and 6 in **II**). Within the clones, 4.4% were 99-100% similar to a *Sphagnum* chloroplast sequence, demonstrating the utilization of $CH_4$ oxidation-derived C by the *Sphagnum* plant.

Table 6. (see next page) Signaling of different phylogenetic *pmoA* probes related to MOB types Ia, Ib and II in the microarray analyses of SAM communities (Y = young, O = old). Signals are normalized relative to the maximum signal level 100 (in black) of the universal reference MOB probe (not included). Table shows average signals, n=1/3/6 (**I**); n=3–8 (**II**) or n=3 (**III**). Probes with only one sample signaling with a signal value less than 2 are not shown. See the more detailed phylogeny of the probes in Abell et al. (2009).

| | | | Type Ia MOB | | | | | | | | | | | | | | Type Ib MOB | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sample | BB51-299 | 511-436 | MbA486 | Mb_SL#3-300 | Mb460 | Mb_C11-403 | Mb271 | DS1_401 | Mm531 | Mm_ES546 | Mm451 | peat_1_3-287 | Mmb562 | LP20-644 | O_la193 | O_la575 | McIT272 | 501-375 | 501-286 | fw1-639 | fw1-641 | LW21-374 | LW21-391 | OSC220 | OSC300 | JRC3-535 | LK580 | M90-253 | Mth413 | lb453 | JR3-505 |
| Study I | Transplantation | Original_0_days | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | Native_0_days | | | | | | | | | 2 | | | | | | 2 | 2 | | | 2 | | | 2 | 3 | | | | | | | |
| | | Immigrant_3_days | | | | | | 1 | | | | | | 1 | | | 1 | 3 | | | | | | | 1 | | | | | | | |
| | | Native_3_days | | | | | | | | | 5 | | | 1 | | | 5 | 5 | | | | | | 3 | 4 | | | | | | | |
| | | Native_28_days | | | | | | | | | 1 | | | 1 | | | 3 | 4 | | | | | | 3 | 2 | | | | | | 1 | |
| | | Immigrant_28_days | | | | | | | | | 1 | 1 | | | | | 3 | 5 | | | | | | 8 | 6 | 2 | 3 | | | | 4 | |
| | | Original_28_days | | | | | | | | | | | | | | | | | | | | | | 6 | 17 | | | | | | | |
| Study II | Un-incubated | Y_wet_meadow | | | 7 | 6 | | 13 | 44 | | 7 | 8 | 13 | | | | 24 | 11 | | 5 | 4 | 3 | 5 | | 8 | | | | | | | |
| | | O_wet_meadow | | | | | | | 2 | | | 10 | 13 | | | | 22 | 19 | | | | 7 | 9 | 8 | 15 | | | | 4 | 4 | | | |
| | | Mesotrophic_fen | | | | | | | 16 | | 3 | 6 | 5 | | | | 18 | 11 | | | | 1 | 3 | 12 | 26 | | 1 | | | 7 | | 3 | |
| | | Oligotrophic_fen | | | | | | | 9 | | 1 | 1 | | | 5 | | 9 | 3 | | | 3 | 4 | 6 | 32 | 60 | | | 2 | 7 | 3 | | 7 | |
| | | Fen-Bog | | | | | | | 6 | | 2 | | 7 | | | | 6 | 6 | | | 2 | 5 | 7 | 31 | 19 | 4 | 2 | 3 | 2 | | | 17 | |
| | Active | Y_wet_meadow | | | | | | | 14 | | | 20 | 20 | | | | 23 | 15 | | | | | | | 6 | | | 3 | | | | | |
| | | O_wet_meadow | | | | | | 1 | | 5 | | 19 | 21 | | | | 22 | 15 | | | | | | 6 | 5 | | | | | | | | |
| | | Mesotrophic_fen | | | | | | | | | | 25 | 25 | | 1 | | 32 | 18 | | | | | | 3 | 7 | | | | | | | | |
| | | Oligotrophic_fen | | | 5 | | | 3 | 13 | | 5 | 4 | 6 | | 5 | | 16 | 21 | | 2 | | | 1 | 26 | 32 | | | | | | | 5 | |
| | | Fen-Bog | | | | | | | 7 | 2 | | 2 | 3 | | 6 | | 12 | 19 | | | | 2 | 3 | 51 | 99 | | | 3 | 2 | | | 5 | |
| Study III | Moss | AN2y | 4 | | | | 2 | | 11 | | 1 | | | | | | 6 | | | | | | | 10 | 5 | | 7 | | | 1 | | 10 | |
| | | AN17y | | 2 | 1 | | | | 2 | | | | | | | | 10 | 8 | 4 | 1 | | | | 13 | 55 | 1 | 5 | | 7 | | | 15 | 4 |
| | | AN63y | | | | | | | | | | | | | | | | | | | | | | | 7 | | | | | | | 2 | |
| | | PRST | | | | | | | | | | | | | | | 3 | 1 | | | | | | 38 | 15 | 8 | 12 | | | | | 16 | |
| | Peat | AN2y | 9 | | | | 2 | | 11 | | | | | 5 | | 1 | 7 | 3 | | | | | | 36 | 82 | | 10 | | | | 2 | 37 | |
| | | AN17y | | | | | | | | | | | | | | 1 | | 4 | | | | | | 21 | 66 | 2 | 2 | | | 8 | | 20 | |
| | | AN63y | | | | | | | | | | | | | | | | | | | | | | 3 | 13 | | | | | | | 3 | |
| | | PRST | | | | | | | | | | | | | | | | | | | | | | 14 | 3 | | 2 | | | | | 4 | |

| | | | Type II MOB | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sample | Mcy233 | Mcy413 | Mcy522 | Mcy270 | Mcy459 | Mcy255 | McyM309 | McyB304 | MsT214 | Msi269 | MsS314 | MsS475 | Msi263 | Msi423 | Msi294 | Msi232 | Peat264 | O_II509 | O_II630 | xb6-539 | LP21-190 | NMsiT-271 | LP21-232 | RA14-591 | B2-400 | B2all343 | B2all341 |
| Study I | Transplantation | Original_0_days | | 8 | 16 | 6 | 18 | | | | | | | | | | | 13 | 52 | 34 | 8 | 6 | 38 | | 3 | | | | |
| | | Native_0_days | 9 | 9 | 19 | 13 | 23 | 1 | 25 | 2 | 4 | | 2 | 8 | | | 2 | 16 | 60 | 42 | 10 | | 5 | 10 | | 15 | | | |
| | | Immigrant_3_days | 3 | 4 | 12 | 7 | 16 | | 6 | 1 | 2 | | 1 | 1 | | | 1 | 12 | 52 | 29 | 7 | | | 5 | | 12 | | | |
| | | Native_3_days | 5 | 7 | 15 | 8 | 19 | | 13 | 1 | 2 | | 1 | 4 | | | 1 | 13 | 54 | 36 | 9 | | 2 | 7 | | 10 | | | |
| | | Native_28_days | 4 | 6 | 11 | 5 | 12 | | 14 | | 1 | | | 4 | | | 1 | 10 | 44 | 20 | 6 | | 1 | 3 | | 7 | | | |
| | | Immigrant_28_days | 6 | 8 | 21 | 12 | 27 | | 18 | 1 | 9 | | | 8 | | | 2 | 18 | 81 | 47 | 12 | | 1 | 6 | | 15 | | | |
| | | Original_28_days | | 1 | 11 | 1 | 15 | | 1 | | 4 | | | | | | | 7 | 53 | 21 | 6 | | | | | 9 | | | |
| Study II | Un-incubated | Y_wet_meadow | 14 | | 8 | | 7 | | | 7 | 29 | | | | | | | 8 | 30 | 10 | 3 | | | 5 | | 14 | | 3 | |
| | | O_wet_meadow | 15 | 7 | 12 | 11 | 17 | 1 | | 20 | 69 | | | 3 | 9 | | | 9 | 62 | 19 | 6 | | 1 | 6 | | 9 | | 19 | 3 |
| | | Mesotrophic_fen | 30 | 5 | 12 | 14 | 11 | | 7 | 27 | 50 | | 2 | 9 | 18 | 4 | 1 | 12 | 36 | 18 | 5 | | | 16 | | 13 | | 6 | 1 |
| | | Oligotrophic_fen | 32 | 7 | 12 | 24 | 13 | 2 | 5 | 11 | 51 | | 1 | 6 | 9 | 1 | 1 | 12 | 45 | 21 | 6 | | | 17 | | 15 | | 13 | 3 |
| | | Fen-Bog | 14 | 6 | 9 | 28 | 8 | 3 | 4 | 5 | 44 | | | | | | 2 | 9 | 36 | 24 | 6 | | | 15 | | 9 | | 10 | 2 |
| | Active | Y_wet_meadow | 4 | | | | 5 | | | | 11 | 6 | | | 6 | | | 5 | 12 | 4 | | | | | | 6 | | 6 | |
| | | O_wet_meadow | | 2 | | 5 | | | | | 8 | | | | 3 | | | 1 | 18 | 1 | | | | | | 1 | | 1 | |
| | | Mesotrophic_fen | 4 | 4 | 5 | | 4 | | 3 | 10 | 19 | | | | | | | 7 | 18 | 4 | | | | 2 | | 5 | | | |
| | | Oligotrophic_fen | 9 | 4 | 5 | 12 | 6 | | | 4 | 31 | | | | | 7 | 1 | 6 | 19 | 8 | 2 | | | 7 | | 8 | 7 | 10 | 4 |
| | | Fen-Bog | 16 | 5 | 5 | 31 | 6 | 2 | 2 | 6 | 26 | | | 3 | | 7 | | 10 | 35 | 21 | 3 | | | 25 | | 9 | | 4 | |
| Study III | Moss | AN2y | 12 | 8 | 18 | 14 | 17 | | 1 | 2 | 18 | | | 3 | 3 | | | 12 | 69 | 33 | 8 | | 2 | 6 | | | | | |
| | | AN17y | 9 | 9 | 8 | 41 | 19 | 7 | 1 | | 1 | | 2 | 2 | | 2 | | 11 | 50 | 35 | 8 | | 6 | 55 | | | | | |
| | | AN63y | 4 | 4 | 8 | 9 | 14 | | | | 1 | | | | 1 | | | 9 | 47 | 18 | 5 | | | | | | | | |
| | | PRST | 4 | 5 | 8 | 6 | 11 | | | 1 | 1 | | | 1 | | | | 11 | 60 | 19 | 5 | | | 4 | | | | | |
| | Peat | AN2y | | 2 | 3 | 7 | 5 | | | 1 | 2 | | | | | | | 6 | 67 | 9 | 2 | 2 | 9 | | 1 | | | | |
| | | AN17y | 15 | 7 | 6 | 30 | 16 | 5 | 1 | | 1 | | | | 11 | | | 9 | 55 | 15 | 5 | | | 12 | | | | | |
| | | AN63y | 1 | 5 | 6 | 9 | 10 | | | | | | | | | | | 11 | 63 | 15 | 4 | | 6 | | | | | | |
| | | PRST | 10 | 7 | 10 | 6 | 12 | | | | 1 | | 2 | 5 | | | | 19 | 70 | 26 | 7 | | 4 | 11 | 5 | | | | |

In addition to the SAM communities, their potential activity was measured before the SIP. All tested 41 moss samples showed detectable MOP. The highest MOP was in the middle phase (mesotrophic fen) and lowest on both ends of the succession gradient, but the MOP rate did not differ significantly between the succession stages (Fig. 5).

The succession stage explained 36.5% of the variation in the community data (all DNA fractions combined) but none of the separate variables (pH, WT or pore water $CH_4$ concentration) had a significant effect on the SAM composition. The *Sphagnum* species did not affect the community diversity or composition (ANOSIM, DNA fractions combined/separate) but had a significant effect on the MOP. However, only *S. platyphyllum* differed significantly from the other *Sphagnum* species. The WT accounted for 18% of the variation in the MOP, whereas the pH or the $CH_4$ concentration did not have an effect. The general SAM diversity did not affect the MOP.

### *4.2.2 SAM on re-vegetating cutover peatlands*

In the third study (**III**), the SAM dynamics were studied in relation to the re-vegetation of the cutover peatlands. The comparison of three re-vegetation stages and one pristine site revealed a successional pattern in both SAM abundance and composition. Based on the qPCR targeting the *pmoA* genes, the SAM belonging to the type II were the most abundant MOB group in all sites and increased with the re-vegetation on the cutover sites (Fig. 4 in **III**). The type I MOB showed an opposite pattern with the type Ia and Ib abundances: they were the highest on the youngest sites restored 2 and 17 years ago (AN2y and AN17y). These successional trends were even more pronounced when compared as relative proportions (Fig. 8). More detailed community composition analysis was done using the *pmoA* gene-based microarray method, which showed a high diversity of type II MOB, particularly *Methylocystis*, on all sites (Table 6 in this summary, Table S2 in **III**). Due to the higher detection limit inherited in this method [5% of the reference probe signal (Bodrossy et al. 2003)], the microarray revealed the type I MOB practically only in one of the three moss samples from the AN2y site. The type Ib diversity seemed to be higher than the type Ia with five or more probes signaling from all sites except AN63y. Similarly to the oldest pristine stages in **II**, the LW21 group-related probes had the strongest type Ib MOB signals among these moss samples. The microarray did not reveal equally clear succession patterns among the SAM communities as the MOB type specific qPCR (Fig. 5B in **III**).

Despite the otherwise high type II MOB diversity, based on the separate PCR analysis of the *Methylocella*-specific *mmox*-genes, this species was either absent or present only in very low numbers in these *Sphagnum* samples (Fig. S4 in **III**).

Based on the MOP measurement, the SAM activity was not tied to the re-vegetation process, as there were no significant differences between the different succession stages (Fig. 5). In comparison to the mosses tested in other studies (**II**, **III**), the MOP of the cutover sites and even on the pristine reference site were on a lower level.

### 4.3 Methane turnover in restored cutover peat layers

In the third study, the SAM analysis was complemented with an examination of the $CH_4$ turnover taking place in the peat layers (10-20 and 20-30 cm below the moss surface) of the re-vegetating cutover sites and the nearby pristine fen used as reference (included also the bottom peat layer). Unlike in the living moss layer, in the peat the MOP and the concurrently measured MPP showed an increase with the re-vegetation succession (Fig. 5 in this summary, Fig. 1 in **III**). The abundances of the *pmoA* types followed a partially similar pattern as seen in the living mosses with total abundances of type Ib and II MOB increasing with the re-vegetation (Fig. 4 in **III**) and the relative abundance of the type Ia simultaneously decreasing (Fig. 8). Accordingly, the *pmoA* microarray showed a slight succession pattern with the youngest AN2y site differing most from the other sites - at least partly due to the higher type Ia diversity (Table 6 in this summary, RDA in Fig. 5a and Table S2 in **III**). Based on the *mmoX*-PCR, *Methylocella* was present at least on the oldest site AN63y and on the pristine site (Fig. S4 in **III**). The re-vegetation also led to an increase in the methanogen abundance (Fig. 2 in **III**), which correlated with the MPP (Table S3 in **III**), and a change in the methanogen community composition (Fig. 3 in **III**). The harsh conditions of the partly vegetated stages (with sedges though) seemed to favor the acetoclastic *Methanosaeta* as these methanogens were detected practically only in the AN2y. In contrast, the hydrogenotrophic methanogens (especially *Methanoregulaceae*) thrived on the more advanced stages. Of the three main functional plant types (Sphagna, sedges, shrubs), Sphagna had the greatest impact on the recovery of the $CH_4$ turnover as all the microbial markers, except the type Ib MOB abundance, correlated with the *Sphagnum* coverage.

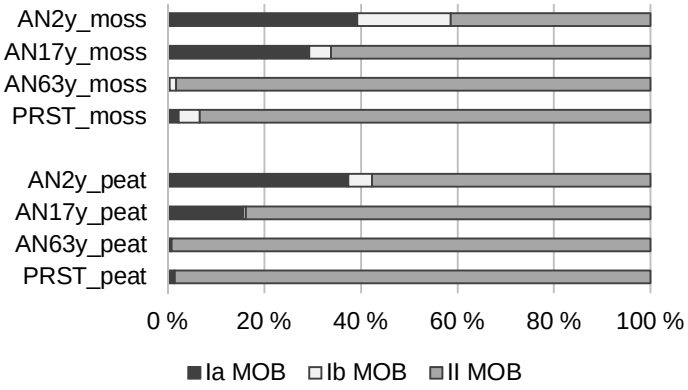


Figure 8. Relative proportions of MOB types in the *Sphagnum* moss and peat (-15 cm layer) samples from the re-vegetated cutover sites, based on the qPCR analysis with MOB type specific primers (n=3). Modified figure S3 from the paper **III**.

# 5 DISCUSSION

## *5.1* Relationship between SAM and *Sphagnum*

Peatlands provide many important ecosystem services. Currently these areas are under various environmental pressures, including the climate change and the more direct anthropogenic disturbances (Andersen et al. 2017; Chimner et al. 2017). Understanding the related impacts and the possible recovery mechanisms is highly important for purposes such as predicting the future C balance and for the evaluation of the best management practices for the utilized peatlands, such as cutover peat basins.

Accordingly, one of the main aims of this thesis was to evaluate how tightly the SAM are connected to the *Sphagnum* mosses. It was based on the theory that obligate, specialized interactions are more vulnerable to disturbances than the facultative ones, as a negative effect on one of the organisms would have a direct impact on the tightly connected symbiont as well. Earlier studies have described the *Sphagnum*-MOB relationship as a mutually beneficial symbiosis as *Sphagnum* is able to use the $CO_2$ formed in the $CH_4$ oxidation especially in the submerged conditions (Kip et al. 2010). In turn, the SAM were believed to benefit from the position inside the moss hyaline cells, which provides more stable conditions than the surrounding water phase. It was also proposed that the SAM are able to use the oxygen formed by the moss photosynthesis (Raghoebarsing et al., 2005; Kip et al., 2010), which has been documented with brown mosses (Liebner et al., 2011; Knoblauch et al. 2015). The results from this thesis support the concept of a mutually beneficial connection but emphasize that at least for the SAM, the connection is not vital for survival. This conclusion of a facultative symbiosis is supported especially by the first study (**I**) which demonstrated that the SAM disperse within the water phase, which allows them to colonize neighboring mosses. Secondly, the SAM composition was not dependent on the *Sphagnum* species, which is in contrast to many other bacterial groups that share their whole life cycle with specific Sphagna (Bragina et al, 2012a; 2013b). Instead of the *Sphagnum* species, SAM were controlled by the more general environmental conditions, as evidenced in both successional studies (**II** and **III**). The loose relationship between the Sphagna and the SAM indicates that the $CH_4$ biofilter provided by the SAM could be relatively resilient towards short-term disturbances such as drought. Due to their ability to colonize the mosses through the water phase, the inactive mosses should regain oxidation activity more efficiently than through the sole re-activation of the "original" SAM community. The movement may be considered to occur not only horizontally, between the living mosses, but also vertically, which would be an important re-activation mechanism after WT drawdown.

Still, based on the study **I** results, the different MOB types may have differences in their connection to the Sphagna. In the transplantation experiment (**I**), many microarray probes signaled in almost all samples, both inactive and active (immigrants/natives) and due to their high prevalence, were impossible to record as "movers". The majority of these probes belonged to the type II MOB, which, despite their potential to move as evidenced by the bathing experiment, may form a "core" SAM community more tightly connected to the mosses. This is supported also by the two other studies (**II** and **III**), where the type II MOB dominated the SAM communities in all pristine (unlabeled DNA) and cutover succession stages except the AN2y. In contrast, the type I MOB were not present in the original inactive mosses (**I**) but appeared only after the transplantation. Especially the type Ia showed clear indications of movement as it was detected only in the mosses of the active transplantation

site (both immigrants and natives). Thus, the type Ia might be more loosely connected to the mosses than the type II MOB.

This thesis did not assess the mechanisms of the detected mobility, but the current knowledge on the motility of MOB types supports the above distinction between the type I and II MOB. Most of the type Ia strains detected in the transplanted mosses and also in the other two studies seemed to be related to the *Methylomonas* species. Although there are non-motile *Methylomonas* strains isolated even from the *Sphagnum* peatlands (Danilova et al. 2013), most of them are capable of active movement with an aid of a single, polar flagellum (Bowman et al. 1993), which appeared to be present also in the (first) *Methylomonas* related strain (M5) isolated from the living *Sphagnum* mosses (Kip et al. 2011b). Similarly, *Methylobacter*, which was detected in some of the thesis samples, contains mostly motile strains (Bowman et al. 1993; exceptions e.g. Wartiainen et al. 2006). In addition, the type Ib MOB contains motile strains, including the first spiral shaped MOB, *Candidatus* Methylospira mobilis, that was recently enriched from a *Sphagnum* peat bog (Danilova et al., 2016) and is detectable by the same probes that signaled in each of the thesis studies (OSC300, LW21-374, LW21-391 and Ib453). In contrast, based on the current knowledge, the dominating type II MOB in these samples, the *Methylocystis*, consists of non-motile strains (Bowman et al. 1993). Thus, most of the type I SAM may have the ability to actively "swim" between the mosses and exhibit a more opportunistic lifestyle, whereas the type II SAM may display a more passive floating-type of movement. Some bacteria, such as the plant-associated methylotrophs, are even able to switch between lifestyles by discarding their flagella after an attachment and biofilm formation on a suitable surface (Kolter and Greenburg, 2006; Doerges et al. 2014). This characteristic has not been detected within the MOB, but then again, their cultivation for example from the living *Sphagnum* mosses is challenging (Kip et al. 2011b).

Overall, the results of this work imply that the moss-SAM relationship is relatively passive and initiated mostly by the favorable conditions inside the hyaline cells and the water as a route for the SAM colonization. However, others have proposed that their interaction might be more complex than just bidirectional transfer of $CO_2$, $O_2$ and, in the case of the diatzotrophic SAM, $N_2$-derived $NH_4^+$, and may involve other microbial groups as intermediates (Ho and Bodelier 2015). In line with this, the SIP experiment (**II**) showed various types of putatively non-MOB bacteria that were using the $^{13}CH_4$-derived C and thus closely connected to the SAM. Among them were methylotrophic species, which are known to be associated with the MOB (Beck et al., 2013). Based on a lake sediment incubation with $CH_4$ as the only C source, Oshkin et al., (2014) reported that only specific methylotrophs/heterotrophs were using MOB-derived C-compounds and thus stated that a passive cross-feeding would not explain their interactions with the MOB. Similarly, in these *Sphagnum* mosses, the $^{13}CH_4$-C seemed to end up only in certain types of bacteria, although based on previous studies the *Sphagnum* microbiome harbors a far wider diversity (Bragina et al. 2012a; 2014). Thus, the SAM may be not only passively feeding the others in the food chain but may be regulated by mechanisms related to these microbial companions.

### 5.2 General patterns in SAM community composition and activity

In this work, the successional patterns of SAM were studied both in pristine (**II**) and restored sites, affected by a severe disturbance (**III**). Despite the obvious differences on these two peatland gradients, they provided similar patterns of ecosystem stability and stress levels:

Due to the lack of a thick peat layer and full *Sphagnum* cover, the young stages were prone to high WT fluctuation that includes repeating cycles of drought and flooding – making the conditions highly unstable (Leppälä et al., 2011). At the same time, the pioneering plants, such as sedges, together with the ground water flow, did provide nutrients and substrates to these systems, which together with the higher pH added up to less stressful conditions. In contrast, especially on the pristine gradient, the older sites with full *Sphagnum* cover provided much more stable hydrological conditions but then again more stress in the form of lower pH and less nutrients. The SAM community compositions changed along these patterns: the young, more dynamic sites with only a partial *Sphagnum* cover seemed to favor the type I MOB and especially the type Ia. These were not only present but dominated the active SAM communities on the three youngest stages of the pristine gradient (**II**) and, even though the cutover study did not include SIP, the relatively high MOP in relation to the high relative type Ia abundance indicated their activity also on the most recently restored AN2y site (**III**). On the other hand, as in the boreal peatlands in general (Dedysh, 2009), the type II MOB were abundant and diverse throughout the gradients but especially on the older, more stable sites with a full *Sphagnum* cover, including the Lakkasuo bog in study **I**. The type Ib related pattern was less clear, as it proved to be active specifically in the older stages (**II**) but showed the highest general abundance on the early re-vegetation stages (**III**). As discussed in **II** and **III**, these patterns fit quite well with the ecological life strategies for the MOB types Ia, Ib and II as proposed by Ho et al. (2013) according to Grime (1977) and as further evidenced by Krause et al. (2014) and Ho et al. (2016). Although the *Sphagnum* species can be separated into similar groups based on their life strategy (Laine et al., 2011), the SAM were not connected to certain *Sphagnum* species. Instead, each MOB type seems to have their preferred ecological niche determined by the wider ecosystem conditions.

The succession-related distribution of the SAM types fits also in with the above discussion of the differences in the motility of the MOB types. The type I MOB who are more capable and disposed to move in the water phase would have an advantage in the young sites with only partial *Sphagnum* cover and highly fluctuating WT whereas less motile type II MOB would be favored by the stable WT level. In addition, although the ability to fix nitrogen is not limited to type II MOB (Knief, 2015), it might aid this MOB type in the ombrotrophic bog stages. In a wider sense, methanotrophy does not seem to enhance *Sphagnum*-associated $N_2$ fixation in the older succession stages (Larmola et al. 2014) and the direct role of MOB within this process remains unclear (Warren et al. 2017).

Interestingly, the SAM activity, as demonstrated by the MOP, showed a different pattern than the SAM community composition: On both gradients (**II** and **III**), the MOP did not change significantly along the succession when stages from the youngest to fen-bog transition were included. Not even the significantly lower *pmoA* abundance, as demonstrated on the youngest cutover site AN2y, had an effect on the potential oxidation activity. This indicates functional redundancy i.e. the same function is fulfilled by multiple groups with each group having their optimum conditions (Naeem, 1998), which is another SAM feature that increases their resilience towards disturbances. Moreover, the high diversity in general has been suggested to increase the ecosystem resilience towards environmental change (Yachi and Loreau 1999; Gunderson 2000). Especially the high active SAM diversity, including the three MOB types Ia, Ib and II and the species not detectable through the *pmoA* (*Methylocella*/*Methyloferula)*, suggests a highly resilient $CH_4$ mitigation particularly on the older succession stages. Redundancy/resilience of the MOB activity is not that surprising as it has been evidenced in peatlands (Peltoniemi et al. 2016), thawing permafrost ponds (Crevecoeur et al. 2017) and in other environments as well: Even though the disturbance

decreased diversity, it did not decrease $CH_4$ oxidation in for example rice fields (Ho et al., 2011) and landfill cover soils (Kumaresan et al., 2011).

The pattern of similar MOP throughout the successional stages broke down with the samples from the oldest bog stages. On the pristine gradient (II), the SAM activity was lowest on the oldest bog site (stage 6) and the same moss samples did not consume enough $^{13}C$-$CH_4$ for the further SIP analysis. In addition, the MOP was not detectable in all mosses from the Lakkasuo bog (**I**). Thus, it seems that considering the $CH_4$ oxidation activity, the fen-bog transition is the crucial step: whereas in the fens the SAM are active in all mosses, in the bogs SAM activity seems to be restricted to the wet microhabitats (flarks) that provide the optimal conditions for the functioning of SAM (Raghoebarsing et al. 2005; Larmola et al. 2010a; Kip et al. 2010; 2011a). In the higher hummocks, the SAM are most likely restricted by the disconnection from the high availability of $CH_4$, which is better available for and also consumed by the MOB living in the peat layer - although the MOP does vary within the microhabitats also in the upper peat (Juottonen et al. 2015). The dominant mosses gathered from the oldest pristine site in **II**, *S. balticum* and *S. fuscum*, did not represent species specialized for submerged conditions, which may partly explain their lower MOP. Overall, the MOP results from all studies (**I**, **II**, **III**) support the previous notion, that the hydrology, especially the long-term WT, is the main controller of the SAM activity.

The measured MOP rates (Fig. 5) were in the lower end of the scale (0 to 62 µmol $g^{-1}$ $day^{-1}$) presented for the Lakkasuo mosses by Larmola et al. (2010a) and especially low in the cutover sites (**III**), including the pristine reference site. The MOP difference between the similar fen-type peatlands may partly be caused by the long-term WT, even though the sampling time WT levels were alike. However, since the sampling times differed [June (**II**) vs. September (**III**)], it may also be due to more general seasonal variation, which ought to be included in the future research designs. In addition, that MOP difference might have been promoted by the different initial $CH_4$ concentrations used in the incubations (see section 3.2).

Unlike the SAM community composition, the SAM activity may be slightly impacted by the *Sphagnum* species as demonstrated by their significant effect on the MOP on the pristine gradient (**II**). However, only one species, *S. platyphyllum* differed significantly from the others. Previous studies have shown contradicting results as Larmola et al. (2010a) reported a larger impact of the total environment than the *S.* species whereas Basiliko et al. (2004) showed significantly different MOP from two different species in the same conditions. Although the cutover study (III) did not specifically consider the effect of *S.* species, it supports the results of Larmola et al. (2010a) since even the bog-type moss *S. balticum* transplanted to the youngest site AN2y showed MOP comparable to other mosses more typical for the fen environment. Moreover, after the transplantation to the wet flark, the *S. rubellum* mosses originating from a drier lawn-microhabitat gained a MOP rate similar to the native flark mosses (**I**).

This thesis used mainly the *pmoA* microarray for the MOB community analysis. The third study utilized also the MOB type specific qPCR, which evidenced the weakness inherited in the otherwise robust microarray method: Due to the low detection limit (5%), small but possibly active groups may be left undetected in environments where one group dominates (type II MOB in this work). Currently, community composition studies are increasingly based on high throughput sequencing methods. To capture all the key members of the community, it is imperative to use methods highlighting the active microbes (RNA, SIP, etc.) or at least to have enough sequencing depth to cover also the smaller groups. Naturally, the PCR-based methods, even when combined with for example SIP, might still leave many important

community members hidden – a limitation that is being overcome through the fast advances in the development of the 'omics' toolbox (Morales and Holben 2010; Prosser 2015).

### 5.3 Succession of SAM in comparison to the $CH_4$ turnover in peat

*Sphagnum*-associated MOB are able to constitute an effective $CH_4$ biofilter for the emissions produced in the peat layers. This has been demonstrated in the field for example by measuring the $CH_4$ flux with and without the moss layer (Frenzel and Karofield 2000; Larmola et al. 2010b). Still, also the MOB in the topmost peat layer are clearly capable of high $CH_4$ mitigation. This thesis evaluated those two components, moss and peat, in parallel, with an aim to reveal their $CH_4$ turnover dynamics during the succession of cutover peatlands.

Compared to the relatively stable conditions within the peat, the mosses on the peat surface are more vulnerable to both short- and long-term environmental changes, such as extreme temperatures and heavy rainfall, which are likely to affect the microbiome of the mosses. On the other hand, the more constant properties of the below peat layers, such as the peat bulk density or even the nutrient status should not affect the moss microbiome as much as it affects the microbes living in the peat. Based on these differences, it was hypothesized (in **III**) that the SAM would not be affected by the re-vegetation succession as much as the peat inhabiting MOB (and methanogens). This held true for the MOP, which stayed similar in the mosses but increased along with the succession in the peat layer. In contrast, the community composition changed both in the Sphagna and in the peat and showed quite similar MOB type patterns. This is not that surprising as, in the end, the *Sphagnum* and peat layers are very closely connected and, as stated above, the MOB most likely move not only horizontally but also vertically with the WT fluctuation. The succession related MOP increase in the peat of these fen-type cutover sites was therefore not related to the MOB community composition but more to the general peat properties and the vegetation succession, which primarily affected the methanogens and, more indirectly, the MOB abundance and activity.

It should be noted, that even as the study III successfully compares the $CH_4$ turnover dynamics between the moss and peat layers, the data does not allow a proper quantitative comparison of the $CH_4$ oxidation activities on an ecosystem scale due to the presentation of the data per mass, not volume. Due to the generally lower density of the mosses, the *in situ* $CH_4$ oxidation in the peat layer is likely larger in relation to the mosses than presented in Fig. 5.

The $CH_4$ turnover has also been studied in the peat layers of the pristine Siikajoki chronosequence, the target of study **III** (Kotiaho et al. 2008; 201x). There, the peat showed higher MPP on the fen sites than on the bogs (based on mass and volume) but the MOP of the topmost peat layer (including living *Sphagnum*) did not change significantly between the sites with over 50% *Sphagnum* cover. Thus, the MOP results from the living *Sphagnum* layer in **II** are quite well in line with the results from Kotiaho et al. (2008; 201x). In the deeper peat layer, on a mass basis, the MOP difference was larger between the fen and bog stages, showing a similar succession-related pattern as the peat layer of the cutover sites in **III**. In addition, other studies on the upper peat, including the living Sphagna, have shown comparable MOP vs. WT patterns: On a WT gradient, the MOP correlated with the *Sphagnum* cover and the higher WT, reflecting the importance of the SAM in the overall MOP (Yrjälä et al. 2011) and in the comparison of microhabitats, the wet hollows showed higher MOP than the hummocks (Juottonen et al. 2015).

In the community analyses that covered both type I and type II MOB, the pristine peat layers have shown community patterns similar to those of the studies **II** (SAM) and **III** (SAM and peat-MOB) with the type I MOB present in the fens and the type II MOB in both fens and bogs (Morris et al. 2002; Jaatinen et al. 2005; Gupta et al. 2012). Moreover, by using SIP with samples from the upper bog layer, Esson et al. (2016) revealed an active MOB composition similar to the SAM communities in **II**.

### 5.4 SAM as a tool in restoration and *Sphagnum* cultivation

While the relatively high SAM-related MOP in the early succession stages (**II**, **III**) may be of less importance on the youngest pristine fens with only partial *Sphagnum* cover, it is of practical value on the peatlands that have undergone anthropogenic disturbances. First, based on this result, it is even more plausible to add *Sphagnum* transplantations to the restoration practices applied on the cutover peatlands – a method that is still more common in North America than in Europe (Andersen et al. 2017; Chimner et al. 2017). While proliferation of the *Sphagnum* requires a WT close to the peat surface (Tuittila et al. 2004), the same conditions also favor the recovery of methanogens and the $CH_4$ production. The results of **III** imply that, in addition to aiding in the C accumulation, the *Sphagnum* mosses and the SAM have the potential to mitigate the $CH_4$ emissions induced by the rewetting/restoration of cutover basins. The *Sphagnum* growth should be actively promoted also on the rewetted forestry-drained peatlands and especially in the blocked ditches, which are known to act as hot pots of $CH_4$ emissions (Minkkinen and Laine 2006; Cooper et al., 2014). The same applies to the peatlands in agricultural use, where the ditches even within the actively farmed areas can show relatively high $CH_4$ emissions (Schrier-Uijl et al. 2010; Luan and Wu 2015). Secondly, the *Sphagnum* mosses are becoming a noteworthy replacement of the pristine peat biomass both as a source of energy and as a horticultural growth medium (Gaudig et al. 2013). For this purpose, the *Sphagnum* mosses are cultivated on areas such as abandoned cutover basins (Beyer and Höper 2015; Pouliot et al. 2015). All these applications are prone to produce $CH_4$ emissions but will most likely benefit from the activity of SAM. While more detailed field-based studies are required to estimate the portion of $CH_4$ potentially blocked by SAM in different settings, this $CH_4$ biofilter characteristic may influence the emission factors that are currently calculated for rewetted organic soils (Hiraishi et al. 2014; Wilson et al. 2016).

As the SAM seem to be able to disperse through the water phase, treating the Sphagna with pore water that shows high MOP might be an effective way to enhance the $CH_4$ biofilter function of the mosses for example in the cutover basins. However, as the transplanted mosses of the recently abandoned cutover site (AN2y) already harbored a diverse SAM community with similar MOP to the older cutover sites, this type of watering might not have any impact on the SAM activity. All that is needed might be a high enough WT, which would control the recovery of the *Sphagnum* mosses (Tuittila et al. 2004; Graf and Rochefort 2010) and the related SAM activity. Still, the incubation-based MOP results do not rule out the possible oxidation enhancing effects gained through regular watering treatments with highly $CH_4$ oxidation active pore water.

# 6 CONCLUSIONS AND FUTURE PERSPECTIVES

The purpose of this thesis was to reveal the ecology of SAM in connection to the succession of their ecosystem, the boreal peatland, and to estimate the impacts of potential disturbances on their functioning. While the SAM community composition was affected by their habitat [succession stage (**II**, **III**) or microhabitat (**I**)], this community response seemed to reflect the redundancy of their $CH_4$ oxidation function as the MOP showed no significant differences between the succession stages, at least within the fen-type peatlands. On the older bog sites, the SAM activity was not always detectable, demonstrating the differences between hydrological microhabitats. Thus, overall, the SAM seemed to have its largest value as a $CH_4$ biofilter in the earlier succession stages, where the Sphagna do not yet form high hummocks above the WT, and on wet bog flarks. Based on these results, when $CH_4$ is present, SAM can to be active in these wetter environments.

The ability to colonize inactive mosses through the water phase was a strong indication of a facultative relationship between the SAM and the *Sphagnum*. Together with the above-mentioned functional redundancy, this trait may provide the SAM and their functioning high resilience towards natural and artificial disturbances, including the predicted climate change-driven increases in extreme weather events (Collins et al. 2013).

The importance of the SAM-related MOP on the fen-type succession stages supports the results of the concurrently made study on SAM induced $N_2$ fixation, which was highest in the minerotrophic fen stage (Larmola et al. 2014). In addition, the demonstrated loose SAM-*Sphagnum* connection fits to the idea that the specificity of a microbial group to certain *Sphagnum* species depends on the function of that group. For example, whereas the SAM are not the only source of $CO_2$ to the mosses, the microbiologically fixed $N_2$ is more essential, showing in the related *nifH* patterns, which are more specific for the *Sphagnum* species (Bragina et al. 2013a). Another main result of this thesis, the relatively high diversity of SAM, was demonstrated also in other simultaneous studies, even on a global scale (Kip et al. 2010; 2011a; 2011c). This thesis complemented those studies in two ways: first, it revealed the potential active members within that SAM diversity. Secondly, it included also the younger fen-type ecosystems, which, despite a substantial *Sphagnum* cover and relatively high $CH_4$ emissions, have not been studied in terms of detailed SAM community composition. As a result, especially the type Ia MOB were shown to be important for the *Sphagnum*-associated $CH_4$ oxidation, particularly in the fens.

Study III was the first, to the author's knowledge, to report SAM dynamics on disturbed peatlands. The analysis of the cutover sites revealed that, despite similar MOB community structures, the $CH_4$ oxidation has the potential to recover faster in the *Sphagnum* mosses than in the peat layer. Thus, SAM can form a valuable $CH_4$ biofilter also on the disturbed sites, which should be taken into account in the restoration practices.

These conclusions should be both further tested and complemented in the future research. This thesis evaluated the SAM activity only through laboratory-based analyzes, which often gives a simplified view on the true *in situ* processes. While the WT seems to be the main controller of the SAM activity, other factors such as temperature, $CH_4$ ebullition/diffusion and even the level of moss photosynthesis may affect SAM activity in the field. In addition, it has to be taken into account that the lack of significant differences between the MOP along the succession gradients may be partly caused by the small sample size that just did not cover the high variability within the different stages. Field-based studies should be used to tackle especially questions related to the predicted climate change. For example, as the climate

warming may induce more long-term droughts, the water mediated recovery of the SAM activity should be evaluated after a more severe and lengthy WT drawdown. Climate change will likely affect the *Sphagnum* cover, both in species composition and in their relative proportion to the other vegetation types, such as vascular plants (Tahvanainen et al. 2011). Through the activity of SAM, the proposed increase in *Sphagnum* cover in the arctic and subarctic regions (Loisel et al. 2012; Gałka et al. 2018) may aid in the mitigation of $CH_4$ emissions induced especially by the thawing permafrost (Johansson et al. 2006; Cooper et al. 2017). However, more detailed analyses, particularly in the spatial scale, are needed to cover both the high variability and complex interactions between the vegetation types and the abiotic factors, already addressed for example by Parmentier et al. (2011). Furthermore, analyses of seasonal and more short-term temporal variation should be employed to define the impact of SAM to the inter-annual variation in wetland-derived $CH_4$ emissions (Ciais et al. 2013).

In general, both MOB and methanogen community data might be valuable in defining the $CH_4$ flux models that are mostly based on abiotic variables (Nazaries et al. 2013). Based on the thesis results, the SAM community composition may not enhance the models, since the *Sphagnum* cover and WT may serve as indicators of the SAM activity. Still, due to differences in their activity levels, the role of different MOB types within the SAM communities deserves further attention. Especially the type Ia MOB abundance may be a useful parameter that, in connection to the above-mentioned factors, could help predict the strength of the *Sphagnum*-associated $CH_4$ biofilter function. In addition, more state of the art methods, namely within the 'omics' approach, should be applied to reveal other microbial groups potentially involved in the $CH_4$ oxidation within *Sphagnum* – either directly or in close association with the SAM. Especially in combination with methods such as SIP, that enable the targeting of a specific function, they would allow the exploration of the *Sphagnum*-associated $CH_4$ oxidation beyond the resolution of the genus or MOB type level presented in this thesis.

This work provided novel insights to a fairly recently discovered phenomenon, the association between the most common plant of the boreal peatlands, the *Sphagnum* moss, and the methanotrophic bacteria. Together they are able to cycle the C, deriving from the degradation of organic matter, directly back to the peat forming biomass, simultaneously mitigating the emissions of the highly effective greenhouse gas, $CH_4$. Based on this thesis, this association is able to both recover from disturbances and adapt to various conditions, and thus has the potential to remain as an effective $CH_4$ biofilter even during environmental change.

# 7 REFERENCES


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. https://doi.org/10.1002/ece3.2469.

Abell G.C.J., Stralis-Pavese N., Sessitsch A., Bodrossy L. (2009). Grazing affects methanotroph activity and diversity in an alpine meadow soil. Environmental Microbiology Reports 1: 457–465. https://doi.org/10.1111/j.1758-2229.2009.00078.x.

Andersen R., Farrell C., Graf M., Muller F., Calvar E., Frankard P., Caporn S., Anderson P. (2017). An overview of the progress and challenges of peatland restoration in Western Europe. Restoration Ecology 25: 1–12. https://doi.org/10.1111/rec.12415.

Angle J.C., Morin T.H., Solden L.M., Angle J.C., Morin T.H., Solden L.M., Narrowe A.B., Smith G.J., Borton M.A., Rey-Sanchez C., Daly R.A., Mirfenderesgi G., Hoyt D.W., Riley W.J. (2017). Methanogenesis in oxygenated soils is a substantial fraction of wetland methane emissions. Nature Communications 8: 1567. https://doi.org/10.1038/s41467-017-01753-4.

Basiliko N., Knowles R., Moore T.R. (2004). Roles of moss species and habitat in methane consumption potential in a northern peatland. Wetlands 24: 178–185. https://doi.org/10.1672/0277-5212(2004)024[0178:ROMSAH]2.0.CO;2.

Basiliko N., Blodau C., Roehm C., Bengtson P., Moore T.R. (2007). Regulation of decomposition and methane dynamics across natural, commercially mined, and restored northern peatlands. Ecosystems 10: 1148–1165. https://doi.org/10.1007/s10021-007-9083-2.

Basiliko N., Henry K., Gupta V., Moore T.R., Driscoll B.T., Dunfield P.F. (2013). Controls on bacterial and archaeal community structure and greenhouse gas production in natural, mined, and restored Canadian peatlands. Frontiers in Microbiology 4: 215. https://doi.org/10.3389/fmicb.2013.00215.

Bateman R.M., Crane P.R., DiMichele W.A., Kenrick P.R., Rowe N.P., Speck T., Stein W.E. (1998). Early evolution of land plants: phylogeny, hysiology, and ecology of the primary terrestrial radiation. Annual Review of Ecology and Systematics 29: 263–292. https://doi.org/10.1146/annurev.ecolsys.29.1.263.

Beck D.A.C., Kalyuzhnaya M.G., Malfatti S., Tringe S.G., del Rio T.G., Ivanova N., Lidstrom M.E., Chistoserdova L. (2013). A metagenomic insight into freshwater methane-utilizing communities and evidence for cooperation between the *Methylococcaceae* and the *Methylophilaceae*. PeerJ 1: e23. https://doi.org/10.7717/peerj.23.

Belova S.E., Baani M., Suzina N.E., Bodelier P.L.E., Liesack W., Dedysh S.N. (2011). Acetate utilization as a survival strategy of peat-inhabiting Methylocystis spp. Environmental Microbiology Reports 3: 36–46. https://doi.org/10.1111/j.1758-2229.2010.00180.x.

Bender M., Conrad R. (1992). Kinetics of $CH_4$ oxidation in oxic soils exposed to ambient air or high CH4 mixing ratios. FEMS Microbiology Letters 101: 261–269. https://doi.org/10.1111/j.1574-6941.1992.tb01663.x.

Benscoter B.W., Wieder R.K. (2003). Variability in organic matter lost by combustion in a boreal bog during the 2001 Chisholm fire. Canadian Journal of Forest Research 2513: 2509–2513. https://doi.org/10.1139/x03-162.

Berg A., Danielsson Å., Svensson B.H. (2013). Transfer of fixed-N from $N_2$-fixing cyanobacteria associated with the moss *Sphagnum riparium* results in enhanced growth of the moss. Plant and Soil 362: 271–278. https://doi.org/10.1007/s11104-012-1278-4.

Beyer C., Höper H. (2015). Greenhouse gas exchange of rewetted bog peat extraction sites and a *Sphagnum* cultivation site in northwest Germany. Biogeosciences 12: 2101–2117. https://doi.org/10.5194/bg-12-2101-2015.

Bodrossy L., Stralis-Pavese N., Murrell J.C., Radajewski S., Weilharter A., Sessitsch A. (2003). Development and validation of a diagnostic microbial microarray for methanotrophs. Environmental Microbiology 5: 566–582. https://doi.org/10.1046/j.1462-2920.2003.00450.x.

Bonn A., Reed M.S., Evans C.D., Joosten H., Bain C., Farmer J., Emmer I., Couwenberg J., Moxey A., Artz R., Tanneberger F. (2014). Investing in nature: Developing ecosystem service markets for peatland restoration. Ecosystem Services 9: 54–65. https://doi.org/10.1016/j.ecoser.2014.06.011.

Bowman J.P., Sly L.I., Nichols P.D., Hayward A.C. (1993). Revised taxonomy of the methanotrophs: Description of *Methylobacter* gen. nov., emendation of *Methylococcus*, validation of *Methylosinus* and *Methylocystis* species, and a proposal that the family *Methylococcaceae* includes only the group I methanotrophs. International Journal of Systematic Bacteriology 43: 735–753. https://doi.org/10.1099/00207713-43-4-735.

Bragina A., Berg C., Cardinale M., Shcherbakov A., Chebotar V., Berg G. (2012a). *Sphagnum* mosses harbour highly specific bacterial diversity during their whole lifecycle. The ISME Journal 6: 802–813. https://doi.org/10.3389/fmicb.2011.00275.

Bragina A., Maier S., Berg C., Müller H., Chobot V., Hadacek F., Berg G. (2012b). Similar diversity of Alphaproteobacteria and nitrogenase gene amplicons on two related *Sphagnum* mosses. Frontiers in Microbiology 2: 275. https://doi.org/10.3389/fmicb.2011.00275.

Bragina A., Berg C., Müller H., Moser D., Berg G. (2013a). Insights into functional bacterial diversity and its effects on Alpine bog ecosystem functioning. Scientific reports 3: 1955. https://doi.org/10.1038/srep01955.

Bragina A., Cardinale M., Berg C., Berg G. (2013b). Vertical transmission explains the specific *Burkholderia* pattern in *Sphagnum* mosses at multi-geographic scale. Frontiers in Microbiology 4: 394. https://doi.org/10.3389/fmicb.2013.00394.

Bragina A., Oberauner-Wappis L., Zachow C., Halwachs B., Thallinger G.G., Müller H., Berg G. (2014). The *Sphagnum* microbiome supports bog ecosystem functioning under extreme conditions. Molecular Ecology 23: 4498–4510. https://doi.org/10.1111/mec.12885.

Bragina A., Berg, C., Berg G. (2015). The core microbiome bonds the Alpine bog vegetation to a transkingdom metacommunity. Molecular Ecology 24: 4795–4807. https://doi.org/10.1111/mec.13342.

Van Breemen N. (1995). How *Sphagnum* bog down other plants. Trends in Ecology & Evolution 10: 270–275. https://doi.org/10.1016/0169-5347(95)90007-1.

Bridgham S.D., Cadillo-Quiroz H., Keller J.K., Zhuang Q. (2013). Methane emissions from wetlands: Biogeochemical, microbial, and modeling perspectives from local to global scales. Global Change Biology 19: 1325–1346. https://doi.org/10.1111/gcb.12131.

Carroll P., Crill P. (1997). Carbon balance of a temperate poor fen. Global Biogeochemical Cycles 11: 349-356. https://doi.org/10.1029/97GB01365.

Chen Y., Dumont M.G., McNamara N.P., Chamberlain P.M., Bodrossy L., Stralis-Pavese N., Murrell J.C. (2008). Diversity of the active methanotrophic community in acidic peatlands as assessed by mRNA and SIP-PLFA analyses. Environmental Microbiology 10: 446–459. https://doi.org/10.1111/j.1462-2920.2007.01466.x.

Chimner R.A., Cooper D.J., Wurster F.C., Rochefort L. (2017). An overview of peatland restoration in North America : where are we after 25 years? Restoration Ecology 25: 283–292. https://doi.org/10.1111/rec.12434.

Ciais P., Sabine C., Bala G., Bopp L., Brovkin V., Canadell J., Chhabra A., DeFries R., Galloway J., Heimann M. and Jones C. (2014). Carbon and other biogeochemical cycles. In: Stocker T.F. et al. (eds.). Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. p. 465–570. https://doi.org/10.1017/CBO9781107415324.

Clymo R.S., Hayward P.M. (1982). The Ecology of *Sphagnum*. In: Smith A.J.E. (ed.) Bryophyte Ecology. Springer Netherlands, Dordrecht. p. 229–289. https://doi.org/10.1007/978-94-009-5891-3_8.

Collins M., Knutti R., Arblaster J., Dufresne J.L., Fichefet T., Friedlingstein P., Gao X., Gutowski W.J., Johns T., Krinner G., Shongwe M. (2013). Long-term climate change: Projections, commitments and irreversibility. In: Stocker T.F. et al. (eds.). Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. p. 1029–1136. https://doi.org/10.1017/CBO9781107415324.

Cooper M.D., Evans C.D., Zielinski P., Levy P.E., Gray A., Peacock M., Norris D., Fenner N., Freeman C. (2014). Infilled ditches are hotspots of landscape methane flux following peatland re-wetting. Ecosystems 17: 1227–1241. https://doi.org/10.1007/s10021-014-9791-3.

Cooper M.D., Estop-Aragonés C., Fisher J.P., Thierry A., Garnett M.H., Charman D.J., Murton J.B., Phoenix G.K., Treharne R., Kokelj S.V., Wolfe S.A. (2017). Limited contribution of permafrost carbon to methane release from thawing peatlands. Nature Climate Change 7: 507–511. https://doi.org/10.1038/nclimate3328.

Costa K.C., Leigh J.A. (2014). Metabolic versatility in methanogens. Current Opinion in Biotechnology 29: 70–75. https://doi.org/10.1016/j.copbio.2014.02.012.

Crevecoeur S, Vincent WF, Comte J, Matveev A, Lovejoy C (2017). Diversity and potential activity of methanotrophs in high methane-emitting permafrost thaw ponds. PLoS ONE 12: 1–22. https://doi.org/10.1371/journal.pone.0188223.

Danilova O.V., Kulichevskaya I.S., Rozova O.N., Detkova E.N., Bodelier P.L.E., Trotsenko Y.A., Dedysh S.N. (2013). *Methylomonas paludis* sp . nov ., the first acid- tolerant member of the genus *Methylomonas* , from an acidic wetland. International Journal of Systematic and Evolutionary Microbiology 63: 2282–2289. https://doi.org/10.1099/ijs.0.045658-0.

Danilova O.V., Suzina N.E., Van De Kamp J., Svenning M.M., Bodrossy L., Dedysh S.N. (2016). A new cell morphotype among methane oxidizers: a spiral-shaped obligately microaerophilic methanotroph from northern low-oxygen environments. The ISME Journal 10: 2734. https://doi.org/10.1038/ismej.2016.48.

Dedysh S.N., Panikov N.S. (1997). Effect of pH, temperature, and concentration of salts on methane oxidation kinetics in *Sphagnum* peat. Microbiology 66: 476-479.

Dedysh S.N., Liesack W., Khmelenina V.N., Suzina N.E., Trotsenko Y.A., Semrau J.D., Bares A.M., Panikov N.S., Tiedje J.M. (2000). *Methylocella palustris* gen. nov., sp. nov., a new methane-oxidizing acidophilic bacterium from peat bogs, representing a novel subtype of serine-pathway methanotrophs. International Journal of Systematic and Evolutionary Microbiology 50: 955–969. https://doi.org/10.1099/00207713-50-3-955.

Dedysh S.N., Derakshani M., Liesack W. (2001). Detection and enumeration of methanotrophs in acidic *Sphagnum* peat by 16S rRNA fluorescence in situ hybridization, including the use of newly developed oligonucleotide probes for *Methylocella palustris*. Applied and Environmental Microbiology 67: 4850–4857. https://doi.org/10.1128/AEM.67.10.4850-4857.2001.

Dedysh S.N., Dunfield P.F., Derakshani M., Stubner S., Heyer J., Liesack W. (2003). Differential detection of type II methanotrophic bacteria in acidic peatlands using newly developed 16S rRNA-

targeted fluorescent oligonucleotide probes. FEMS Microbiology Ecology 43: 299–308. https://doi.org/10.1111/j.1574-6941.2003.tb01070.x.

Dedysh S.N., Knief C., Dunfield P.F. (2005). *Methylocella* species are facultatively methanotrophic. Journal of Bacteriology 187: 4665–4670. https://doi.org/10.1128/JB.187.13.4665-4670.2005.

Dedysh S.N., Pankratov T.A., Belova S.E., Kulichevskaya I.S., Liesack W. (2006). Phylogenetic analysis and in situ identification of bacteria community composition in an acidic *Sphagnum* peat bog. Applied and Environmental Microbiology 72: 2110–2117. https://doi.org/10.1128/AEM.72.3.2110-2117.2006.

Dedysh S.N. (2009). Exploring methanotroph diversity in acidic northern wetlands: Molecular and cultivation-based studies. Microbiology 78: 655–669. https://doi.org/10.1134/S0026261709060010.

Doerges L., Kutschera U. (2014). Assembly and loss of the polar flagellum in plant-associated methylobacteria. Naturwissenschaften 101: 339–346. https://doi.org/10.1007/s00114-014-1162-6.

Dumont M.G., Lüke C., Deng Y., Frenzel P. (2014). Classification of *pmoA* amplicon pyrosequences using BLAST and the lowest common ancestor method in MEGAN. Frontiers in Microbiology 5: 34. https://doi.org/10.3389/fmicb.2014.00034.

Dunfield P., Dumont R., Moore, T.R. (1993). Methane production and consumption in temperate and subarctic peat soils: response to temperature and pH. Soil Biology and Biochemistry 25: 321-326. https://doi.org/10.1016/0038-0717(93)90130-4.

Dunfield P.F., Yurye, A., Senin P., Smirnova A.V., Stott M.B., Hou S., Ly, B., Saw J.H., Zhou Z., Ren Y., Wang J. (2007). Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. Nature 450: 879–882. https://doi.org/10.1038/nature06411.

Van Den Elzen E., Kox M.A., Harpenslager S.F., Hensgens G., Fritz C., Jetten M.S., Ettwig K.F., Lamers L.P. (2017). Symbiosis revisited: Phosphorus and acid buffering stimulate $N_2$ fixation but not *Sphagnum* growth. *Biogeosciences* 14: 1111–1122. https://doi.org/10.5194/bg-14-1111-2017.

Esson K.C., Lin X., Kumaresan D., Chanton J.P., Murrell J.C., Kostka E., Kostka J.E. (2016). Alpha- and gammaproteobacterial methanotrophs codominate the active methane-oxidizing communities in an acidic boreal peat bog. Applied and Environmental Microbiology 82: 2363–2371. https://doi.org/10.1128/AEM.03640-15.

Etminan M., Myhre G., Highwood E.J., Shine K.P. (2016). Radiative forcing of carbon dioxide, methane, and nitrous oxide: A significant revision of the methane radiative forcing. Geophysical Research Letters 43: 12,614-12,623. https://doi.org/10.1002/2016GL071930.

Ettwig K.F., Zhu B., Speth D., Keltjens J.T., Jetten M.S.M., Kartal B. (2016). Archaea catalyze iron-dependent anaerobic oxidation of methane. Proceedings of the National Academy of Sciences 113: 12792–12796. https://doi.org/10.1073/pnas.1609534113.

Evans P.N., Parks D.H., Chadwick G.L., Robbins S.J., Orphan V.J., Golding S.D., Tyson G.W. (2015). Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics. Science 350: 434–8. https://doi.org/10.1126/science.aac7745.

Francez A.J., Gogo S., Josselin N. (2000). Distribution of potential $CO_2$ and $CH_4$ productions, denitrication and microbial biomass C and N in the the profile of a restored peatland in Brittany (France). European Journal of Soil Biology 36: 161–168. https://doi.org/10.1016/S1164-5563(00)01057-8.

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. https://doi.org/10.1023/A:1006351118347.

Fritz C., Pancotto V.A., Elzenga J., Visser E.J., Grootjans A.P., Pol A., Iturraspe R., Roelofs J.G., Smolders A.J. (2011). Zero methane emission bogs: Extreme rhizosphere oxygenation by cushion

plants in Patagonia. New Phytologist 190: 398–408. https://doi.org/10.1111/j.1469-8137.2010.03604.x.

Galand P.E., Juottonen H., Fritze H., Yrjälä K. (2005). Methanogen communities in a drained bog: Effect of ash fertilization. Microbial Ecology 49: 209–217. https://doi.org/10.1007/s00248-003-0229-2.

Gałka M., Tanţău I., Feurdean A. (2017). Plant succession in a peatland in the Eastern Carpathian Mts. (CE Europe) during the last 10,200 years: Implications for peatland development and palaeoclimatic research. Review of Palaeobotany and Palynology 244: 203–216. https://doi.org/10.1016/j.revpalbo.2017.05.014.

Gałka M., Swindles G.T., Szal M., Fulweber R., Feurdean A. (2018). Response of plant communities to climate change during the late Holocene: Palaeoecological insights from peatlands in the Alaskan Arctic. Ecological Indicators 85: 525–536. https://doi.org/10.1016/j.ecolind.2017.10.062.

Gaudig G., Fengler F., Krebs M., Prager A., Schulz J., Wichmann S., Joosten H. (2013). *Sphagnum* farming in Germany – a review of progress. Mires and Peat 13: 1–11.

Glatzel S,. Basiliko N., Moore T., Unit L.E. (2004). Carbon dioxide and methane production potentials of peats from natural , harvested and restored sites. Wetlands 24: 261–267. https://doi.org/10.1672/0277-5212(2004)024[0261:CDAMPP]2.0.CO;2.

Gorham E. (1991). Role in the carbon cycle and probable responses to climatic warming. Ecological Applications 1: 182–195. https://doi.org/10.2307/1941811.

Graf M.D., Rochefort L. (2010). Moss regeneration for fen restoration: Field and greenhouse experiments. Restoration Ecology 18: 121–130. https://doi.org/10.1111/j.1526-100X.2008.00437.x.

Grime J.P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. The American Naturalist 111: 1169–1194. https://doi.org/10.1086/283244.

Gunderson L.H. (2000). Ecological resilience- in theory and application. Annual Review of Ecology and Systematics 31: 425–39. https://doi.org/10.1146/annurev.ecolsys.31.1.425.

Gupta V., Smemo K.A., Yavitt J.B., Basiliko N. (2012). Active methanotrophs in two contrasting north american peatland ecosystems revealed using DNA-SIP. Microbial Ecology 63: 438–445. https://doi.org/10.1007/s00248-011-9902-z.

Gupta V., Smemo K.A., Yavitt J.B., Fowle D., Branfireun B., Basiliko N. (2013). Stable isotopes reveal widespread anaerobic methane oxidation across latitude and peatland type. Environmental Science and Technology 47: 8273–8279. https://doi.org/10.1021/es400484t.

Hájek T., Ballance S., Limpens J., Zijlstra M., Verhoeven J.T.A. (2011). Cell-wall polysaccharides play an important role in decay resistance of *Sphagnu*m and actively depressed decomposition in vitro. Biogeochemistry 103: 45–57. https://doi.org/10.1007/s10533-010-9444-3.

Hanson R.S., Hanson T.E. (1996). Methanotrophic bacteria. Microbiological reviews 60: 439–471.

Hardoim P.R., van Overbeek L.S., Elsas J.D. van (2008). Properties of bacterial endophytes and their proposed role in plant growth. Trends in Microbiology 16: 463–471. https://doi.org/10.1016/j.tim.2008.07.008.

Hardy R.W.F., Holsten R.D., Jackson E.K., Burns R.C. (1968). The acetylene - ethylene assay for $N_2$ fixation: laboratory and field evaluation. Plant physiology 43: 1185–1207. https://doi.org/10.1104/pp.43.8.1185.

Hartmann D.L., Tank A.M.K., Rusticucci M., Alexander L.V., Brönnimann S., Charabi Y.A.R., Dentener F.J., Dlugokencky E.J., Easterling D.R., Kaplan A., Soden B.J. (2013). Observations: Atmosphere and Surface. In: Stocker T.F. et al. (eds.). Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental

Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. p. 159–254. https://doi.org/10.1017/CBO9781107415324.

Hines M.E., Duddleston K.N., Rooney-Varga J.N., Fields D., Chanton J.P. (2008). Uncoupling of acetate degradation from methane formation in Alaskan wetlands: Connections to vegetation distribution. Global Biogeochemical Cycles 22: 1–12. https://doi.org/10.1029/2006GB002903.

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

Ho A., Lüke C., Frenzel P. (2011). Recovery of methanotrophs from disturbance: population dynamics, evenness and functioning. The ISME journal 5: 750–8. https://doi.org/10.1038/ismej.2010.163.

Ho A., Kerckhof F.M., Luke C., Reim A., Krause S., Boon N., Bodelier P.L.E. (2013). Conceptualizing functional traits and ecological characteristics of methane-oxidizing bacteria as life strategies. Environmental Microbiology Reports 5: 335–345. https://doi.org/10.1111/j.1758-2229.2012.00370.x.

Ho A., Bodelier P.L.E. (2015). Diazotrophic methanotrophs in peatlands: the missing link? Plant and Soil 389: 419–423. https://doi.org/10.1007/s11104-015-2393-9.

Ho A., Brink E. Van Den, Reim A., Krause S.M.B., Nicol G.W., Dumont M.G. (2016). Recurrence and frequency of disturbance have cumulative effect on methanotrophic activity, abundance, and community structure. Frontiers in Microbiology 6: 1493. https://doi.org/10.3389/fmicb.2015.01493.

Hughes P.D.M., Barber K.E. (2004). Contrasting pathways to ombrotrophy in three raised bogs from Ireland and Cumbria, England. Holocene 14: 65–77. https://doi.org/10.1191/0959683604hl690rp.

Im J., Lee S.W., Yoon S., Dispirito A.A., Semrau J.D. (2011). Characterization of a novel facultative *Methylocystis* species capable of growth on methane, acetate and ethanol. Environmental Microbiology Reports 3: 174–181. https://doi.org/10.1111/j.1758-2229.2010.00204.x.

Islam T., Jensen S., Reigstad L.J., Larsen O., Birkeland N.-K. (2008). Methane oxidation at 55 °C and pH 2 by a thermoacidophilic bacterium belonging to the Verrucomicrobia phylum. Proceedings of the National Academy of Sciences 105: 300–304. https://doi.org/10.1073/pnas.0704162105.

Jaatinen K., Tuittila E.-S., Laine J., Yrjälä K., Fritze H. (2005). Methane-oxidizing bacteria in a finnish raised mire complex: effects of site fertility and drainage. Microbial Ecology 50: 429–439. https://doi.org/10.1007/s00248-005-9219-x.

Jassey V.E.J., Gilbert D., Binet P., Toussaint M.-L., Chiapusio G. (2011). Effect of a temperature gradient on *Sphagnum fallax* and its associated living microbial communities: a study under controlled conditions. Canadian journal of microbiology 57: 226–235. https://doi.org/10.1139/W10-116.

Jassey V.E., Chiapusio G., Binet P., Buttler A., Laggoun-Défarge F., Delarue F., Bernard N., Mitchell E.A., Toussaint M.L., Francez A.J., Gilbert D. (2013). Above- and belowground linkages in *Sphagnum* peatland: Climate warming affects plant-microbial interactions. Global Change Biology 19: 811–823. https://doi.org/10.1111/gcb.12075.

Johansson T., Malmer N., Crill P.M., Friborg T., Åkerman J.H., Mastepanov M., Christensen T.R. (2006). Decadal vegetation changes in a northern peatland, greenhouse gas fluxes and net radiative forcing. Global Change Biology 12: 2352–2369. https://doi.org/10.1111/j.1365-2486.2006.01267.x.

Johnson M.G., Granath G., Tahvanainen T., Pouliot R., Stenøien H.K., Rochefort L., Rydin H., Shaw A.J. (2015). Evolution of niche preference in *Sphagnum* peat mosses. Evolution 69: 90–103. https://doi.org/10.1111/evo.12547.

Juottonen H., Galand P.E., Tuittila E.-S., 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. https://doi.org/10.1111/j.1462-2920.2005.00838.x.

Juottonen H., Hynninen A., Nieminen M., Tuomivirta T.T., Tuittila E.S., Nousiainen H., Kell D.K., 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. https://doi.org/10.1128/AEM.00261-12.

Juottonen H., Kotiaho M., Robinson D., Merilä P., Fritze H., Tuittila E.-S. (2015). Microform-related community patterns of methane-cycling microbes in boreal *Sphagnum* bogs are site specific. FEMS Microbiology Ecology 91: 1–13. https://doi.org/10.1093/femsec/fiv094.

Juottonen H., Eiler A., Biasi C., Tuittila E.-S., Yrjälä K., Fritze H. (2017). Distinct anaerobic bacterial consumers of cellobiose-derived carbon in boreal fens with different $CO_2/CH_4$ production ratios. Applied and Environmental Microbiology 83: e02533-16. https://doi.org/10.1128/AEM.02533-16.

Keppler F., Hamilton J.T., Bra M., Rockmann T. (2006). Methane emissions from terrestrial plants under aerobic conditions. Nature 439: 187–191. https://doi.org/10.1038/nature04420.

Kettunen A., Kaitala V., Lehtinen A., Lohila A., Alm J., Silvola J., Martikainen P.J. (1999). Methane production and oxidation potentials in relation to water table fluctuations in two boreal mires. Soil Biology and Biochemistry 31: 1741–1749. https://doi.org/10.1016/S0038-0717(99)00093-0.

Kip N., Van Winden J.F., Pan Y., Bodrossy L., Reichart G.J., Smolders A.J., Jetten M.S., Damsté J.S.S., Op den Camp H.J.M. (2010). Global prevalence of methane oxidation by symbiotic bacteria in peat-moss ecosystems. Nature Geoscience 3: 617–621. https://doi.org/10.1038/ngeo939.

Kip N., Fritz,C., Langelaan E.S., Pan Y., Bodrossy L., Pancotto V., Jetten M.S.M., Smolders A.J.P., Op den Camp H.J.M. (2011a). Methanotrophic activity and diversity in different *Sphagnum magellanicum* dominated habitats in the southernmost peat bogs of Patagonia. Biogeosciences 9: 47–55. https://doi.org/10.5194/bg-9-47-2012.

Kip N., Ouyang W., van Winden J., Raghoebarsing A., van Niftrik L., Pol A., Pan Y., Bodrossy L., van Donselaar E.G., Reichart G.J., Jetten M.S. (2011b). Detection, isolation, and characterization of acidophilic methanotrophs from *Sphagnum* mosses. Applied and Environmental Microbiology 77: 5643–5654. https://doi.org/10.1128/AEM.05017-11.

Kip N., Dutilh B.E., Pan Y., Bodrossy L., Neveling K., Kwint M.P., Jetten M.S., Op den Camp H.J.M. (2011c). Ultra-deep pyrosequencing of *pmoA* amplicons confirms the prevalence of *Methylomonas* and *Methylocystis* in *Sphagnum* mosses from a Dutch peat bog. Environmental Microbiology Reports 3: 667–673. https://doi.org/10.1111/j.1758-2229.2011.00260.x.

Klinger L.F., Short S.K. (1996). Succession in the hudson bay lowland, Northern Ontario, Canada. Arctic and Alpine Research 28: 172–183. https://doi.org/10.2307/1551757.

Knief C. (2015). Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on *pmoA* as molecular marker. Frontiers in Microbiology 6: 1346. https://doi.org/10.3389/fmicb.2015.01346.

Knoblauch C., Spott O., Evgrafova S., Kutzbach L., Pfeiffer E.M. (2015). Regulation of methane production, oxidation, and emission by vascular plants and bryophytes in ponds of the northeast Siberian polygonal tundra. Biogeosciences 120: 2525–2541. https://doi.org/10.1002/2015JG003053.

Kolter R., Greenberg E.P. (2006). Microbial sciences: The superficial life of microbes. Nature 441: 300–302. https://doi.org/10.1038/441300a.

Komulainen V., Tuittila E.-S. (1999). Restoration of drained peatlands in southern Finland : initial effects on vegetation change and $CO_2$ balance. Journal of Applied Ecology 36: 634–648. https://doi.org/10.1046/j.1365-2664.1999.00430.x.

Korrensalo A., Alekseychik P., Hájek T., Rinne J., Vesala T., Mehtätalo L. (2017). Species-specific temporal variation in photosynthesis as a moderator of peatland carbon sequestration. Biogeosciences 14: 257–269. https://doi.org/10.5194/bg-14-257-2017.

Kostka J.E., Weston D.J., Glass J.B., Lilleskov E.A., Shaw A.J., Turetsky M.R. (2016). The *Sphagnum* microbiome: New insights from an ancient plant lineage. New Phytologist 211: 57–64. https://doi.org/10.1111/nph.13993.

Kotiaho M., Fritze H., Merilä P., Leppälä M., Juottonen H., Putkinen A., Sarjala T., Tuittila E.-S. (2008). Successional patterns of in vitro soil respiration and methane production and oxidation along a mire chronosequence. In: Farrell, C., Feehan, J. (eds.). Proceedings of the 13th International Peat Congress. After Wise Use-The Future of Peatlands. Abstracts of Presentations. Tullamore, Ireland, 8-13 June 2008. p. 3470.

Kotiaho M., Fritze H., Merilä P., Juottonen H., Leppälä M., Laine J., Laiho R., Yrjälä K., Tuittila E.-S. (2010). Methanogen activity in relation to water table level in two boreal fens. Biology and Fertility of Soils 46: 567–575. https://doi.org/10.1007/s00374-010-0461-0.

Kotiaho M., Hamberg L., Laine A., Putkinen A., Fritze H., Merilä P., Peltoniemi K., Tuittila E.-S., Juottonen H. (201x). Microbial change along a mire development gradient. *Manuscript in progress*.

Kotsyurbenko O.R., Friedrich M.W., Simankova M.V., Nozhevnikova A.N., Golyshin P.N., Timmis K.N., Conrad R. (2007). Shift from acetoclastic to $H_2$-dependent methanogenesis in a west Siberian peat bog at low pH values and isolation of an acidophilic *Methanobacterium* strain. Applied and Environmental Microbiology 73: 2344–2348. https://doi.org/10.1128/AEM.02413-06.

Krause S., Le Roux X., Niklaus P.A., Van Bodegom P.M., Lennon J.T., Bertilsson S., Grossart H.P., Philippot L., Bodelier P.L. (2014). Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. Frontiers in Microbiology 5: 251. https://doi.org/10.3389/fmicb.2014.00251.

Kumaresan D., Stralis-Pavese N., Abell G.C.J., Bodrossy L., Murrell J.C. (2011). Physical disturbance to ecological niches created by soil structure alters community composition of methanotrophs. Environmental Microbiology Reports 3: 613–621. https://doi.org/10.1111/j.1758-2229.2011.00270.x.

Laine J., Komulainen V.M., Laiho R., Minkkinen K., Rasinmäki A., Sallantaus T., Sarkkola S., Silvan N., Tolonen K., Tuittila E.-S., Vasander H. (2004). Lakkasuo - a guide to mire ecosystem. University of Helsinki Department of Forest Ecology Publications 31. 123 p.

Laine A.M., Juurola E., Hájek T., Tuittila E.-S. (2011). *Sphagnum* growth and ecophysiology during mire succession. Oecologia 167: 1115–1125. https://doi.org/10.1007/s00442-011-2039-4.

Laine A.M., Bubier J.L., Riutta T., Nilsson M.B., Moore T.R., Vasander H., Tuittila E.-S. (2012). Abundance and composition of plant biomass as potential controls for mire net ecosytem $CO_2$ exchange. Botany 90: 63–74. https://doi.org/10.1139/b11-068.

Laine A.M., Tolvanen A., Mehtätalo L., Tuittila E.-S. (2016). Vegetation structure and photosynthesis respond rapidly to restoration in young coastal fens. Ecology and Evolution 6: 6880–6891. https://doi.org/10.1002/ece3.2348.

Lang K., Schuldes J., Klingl A., Poehlein A., Daniel R., Brune A. (2015). New mode of energy metabolism in the seventh order of methanogens as revealed by comparative genome analysis of "Candidatus *Methanoplasma termitum*." Applied and Environmental Microbiology 81: 1338–1352. https://doi.org/10.1128/AEM.03389-14.

Larmola T., Tuittila E.S., Tiirola M., Nykänen H., Martikainen P.J., Yrjälä K., Tuomivirta T., Fritze H. (2010a). The role of *Sphagnum* mosses in the methane cycling of a boreal mire. Ecology 91: 2356–2365. https://doi.org/10.1890/09-1343.1.

Larmola T., Koponen H., Riutta T., Fritze H., Goodrich J., Varner R., Bubier J., Juutinen S., Rinne J., Vesala T., Martikainen P.J. (2010b). *Sphagnum* mosses as methane traps in two northern mires. In: EGU General Assembly Conference Abstracts 12. p. 8833.

Larmola T., Leppänen S.M., Tuittila E.-S., Aarva M., Merilä P., Fritze H., Tiirola M. (2014). Methanotrophy induces nitrogen fixation during peatland development. Proceedings of the National Academy of Sciences 111: 734–739. https://doi.org/10.1073/pnas.1314284111.

Lenhart K., Bunge M., Ratering S., Neu T.R., Schüttmann I., Greule M., Kammann C., Schnell S., Müller C., Zorn H., Keppler F. (2012). Evidence for methane production by saprotrophic fungi. Nature Communications 3: 1046. https://doi.org/10.1038/ncomms2049.

Leppälä M., Kukko-Oja K., Laine .J, Tuittila E.-S. (2008). Seasonal dynamics of $CO_2$ exchange during primary succession of boreal mires as controlled by phenology of plants. Ecoscience 15: 460–471. https://doi.org/10.2980/15-4-3142.

Leppälä M., Oksanen J., Tuittila E.-S. (2011). Methane flux dynamics during mire succession. Oecologia 165: 489–499. https://doi.org/10.1007/s00442-010-1754-6.

Leppänen S.M., Rissanen A.J., Tiirola M. (2015). Nitrogen fixation in *Sphagnum* mosses is affected by moss species and water table level. Plant and Soil 389: 185–196. https://doi.org/10.1007/s11104-014-2356-6.

Liebner S., Zeyer J., Wagner D., Schubert C., Pfeiffer E.M., Knoblauch C. (2011). Methane oxidation associated with submerged brown mosses reduces methane emissions from Siberian polygonal tundra. Journal of Ecology 99: 914–922. https://doi.org/10.1111/j.1365-2745.2011.01823.x.

Liebner S., Svenning M.M. (2013). Environmental transcription of *mmoX* by methane-oxidizing Proteobacteria in a subarctic palsa peatland. Applied and Environmental Microbiology 79: 701–706. https://doi.org/10.1128/AEM.02292-12.

Liebner S., Ganzert L., Kiss A., Yang S., Wagner D., Svenning M.M. (2015). Shifts in methanogenic community composition and methane fluxes along the degradation of discontinuous permafrost. Frontiers in Microbiology 6: 356. https://doi.org/10.3389/fmicb.2015.00356.

Liu H., Carvalhais L.C., Crawford M., Singh E., Dennis P.G., Pieterse C.M.J., Schenk P.M. (2017). Inner plant values: diversity, colonization and benefits from endophytic bacteria. Frontiers in Microbiology 8: 2552. https://doi.org/10.3389/fmicb.2017.02552.

Loisel J., Gallego-Sala A.V., Yu Z. (2012). Global-scale pattern of peatland *Sphagnum* growth driven by photosynthetically active radiation and growing season length. Biogeosciences 9: 2737–2746. https://doi.org/10.5194/bg-9-2737-2012.

Luan J., Wu J. (2015). Long-term agricultural drainage stimulates $CH_4$ emissions from ditches through increased substrate availability in a boreal peatland. Agriculture, Ecosystems and Environment 214: 68–77. https://doi.org/10.1016/j.agee.2015.08.020.

Luton P.E., Wayne J.M., Sharp R.J., Riley P.W. (2002). The *mcrA* gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. Microbiology 148: 3521–3530. https://doi.org/10.1099/00221287-148-11-3521.

Maanavilja L., Aapala K., Haapalehto T., Kotiaho J.S., Tuittila E.-S. (2014). Impact of drainage and hydrological restoration on vegetation structure in boreal spruce swamp forests. Forest Ecology and Management 330: 115–125. https://doi.org/10.1016/j.foreco.2014.07.004.

Macdonald G.M., Beilman D.W., Kremenetski K.V., Sheng Y., Smith L.C., Velichko A. (2006). Rapid early development of circumarctic peatlands and atmospheric $CH_4$ and $CO_2$ variations. Science 314: 285–288. https://doi.org/10.1126/science.1131722.

Machacova K., Bäck J., Vanhatalo A., Halmeenmäki E., Kolari P., Mammarella I., Pumpanen J., Acosta M., Urban O., Pihlatie M. (2016). *Pinus sylvestris* as a missing source of nitrous oxide and methane in boreal forest. Scientic Reports 6: 23410. https://doi.org/10.1038/srep23410.

Maljanen M., Sigurdsson B.D., Guðmundsson J., Óskarsson H., Huttunen J.T., Martikainen P.J. (2010). Greenhouse gas balances of managed peatlands in the Nordic countries – present knowledge and gaps. Biogeosciences 2: 2711–2738. https://doi.org/10.5194/bg-7-2711-2010.

Martikainen P.J., Nykänen H., Alm J., Silvola J. (1995). Change in fluxes of carbon-dioxide, methane and nitrous-oxide due to forest drainage of mire sites of different trophy. Plant and Soil 168: 571–577. https://doi.org/10.1007/BF00029370.

Mastný J., Urbanová Z., Kaštovská E., Straková P., Šantrůčková H., Edwards K.R., Picek T. (2016). Soil organic matter quality and microbial activities in spruce swamp forests affected by drainage and water regime restoration. Soil Use and Management 32: 200–209. https://doi.org/10.1111/sum.12260.

McDonald I.R., Bodrossy L., Chen Y., Murrell J.C. (2008). Molecular ecology techniques for the study of aerobic methanotrophs. Applied and Environmental Microbiology 74: 1305–1315. https://doi.org/10.1128/AEM.02233-07.

McNeil P., Waddington J.M. (2003). Moisture controls on *Sphagnum* growth and $CO_2$ exchange on a cutover bog. Journal of Applied Ecology 40: 354–367. https://doi.org/10.1046/j.1365-2664.2003.00790.x.

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. https://doi.org/10.1016/S1164-5563(01)01067-6.

Merilä P., Galand P.E., Fritze H., Tuittila E.-S., Kukko-Oja K., Laine J., Yrjälä K. (2006). Methanogen communities along a primary succession transect of mire ecosystems. FEMS Microbiology Ecology 55: 221–229. https://doi.org/10.1111/j.1574-6941.2005.00030.x.

Michaelis W., Seifert R., Nauhaus K., Treude T., Thiel V., Blumenberg M., Knittel K., Gieseke A., Peterknecht K., Pape T., Boetius A. (2002). Microbial reefs in the black sea fueled by anerobic oxidation of Methane. Science 297: 1013–1015. https://doi.org/10.1126/science.1072502.

Minkkinen K., Laine J. (2006). Vegetation heterogeneity and ditches create spatial variability in methane fluxes from peatlands drained for forestry. Plant and Soil 285: 289–304. https://doi.org/10.1007/s11104-006-9016-4.

Mondav R., Woodcroft B.J., Kim E.H., McCalley C.K., Hodgkins S.B., Crill P.M., Chanton J., Hurst G.B., VerBerkmoes N.C., Saleska S.R., Hugenholtz P. (2014). Discovery of a novel methanogen prevalent in thawing permafrost. Nature communications 5: 3212. https://doi.org/10.1038/ncomms4212.

Morris S.A., Radajewski S., Willison T.W., Murrell J.C. (2002). Identification of the functionally active methanotroph population in a peat soil microcosm by stable-isotope probing. Applied and Environmental Microbiology 68: 1446–1453. https://doi.org/10.1128/AEM.68.3.1446-1453.2002.

Morales S.E., Holben W.E. (2011). Linking bacterial identities and ecosystem processes: can 'omic'analyses be more than the sum of their parts? FEMS microbiology ecology 75: 2-16. https://doi.org/10.1111/j.1574-6941.2010.00938.x.

Myhre G., Shindell D., Bréon F.M., Collins W., Fuglestvedt J., Huang J., Koch D., Lamarque J.F., Lee D., Mendoza B., Nakajima T. (2013). Anthropogenic and Natural Radiative Forcing. In: Stocker T.F. et al. (eds.). Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. p. 659–740. https://doi.org/10.1017/CBO9781107415324.

Naeem S. (1998). Species Redundancy and Ecosystem Reliability. Conservation Biology 12: 39–45. https://doi.org/10.1046/j.1523-1739.1998.96379.x.

Nazaries L., Murrell J.C., Millard P., Baggs L., Singh B.K. (2013). Methane, microbes and models: Fundamental understanding of the soil methane cycle for future predictions. Environmental Microbiology 15: 2395–2417. https://doi.org/10.1111/1462-2920.12149.

Ojanen P., Minkkinen K., Penttilä T. (2013). The current greenhouse gas impact of forestry-drained boreal peatlands. Forest Ecology and Management 289: 201–208. https://doi.org/10.1016/j.foreco.2012.10.008.

Op den Camp H.J., Islam T., Stott M.B., Harhangi H.R., Hynes A., Schouten S., Jetten M.S., Birkeland N.K., Pol A., Dunfield P.F., (2009). Environmental, genomic and taxonomic perspectives on methanotrophic Verrucomicrobia. Environmental Microbiology Reports 1: 293–306. https://doi.org/10.1111/j.1758-2229.2009.00022.x.

Opelt K., Berg G. (2004). Diversity and antagonistic potential of bacteria associated with bryophytes from nutrient-poor habitats of the Baltic sea coast diversity and antagonistic potential of bacteria associated with bryophytes from nutrient-poor habitats of the Baltic Sea Coast. Applied and environmental microbiology 70: 6569–6579. https://doi.org/10.1128/AEM.70.11.6569-6579.2004.

Opelt K., Berg C., Schönmann S., Eberl L., Berg G. (2007a). High specificity but contrasting biodiversity of *Sphagnum*-associated bacterial and plant communities in bog ecosystems independent of the geographical region. The ISME journal 1: 502–16. https://doi.org/10.1038/ismej.2007.58.

Opelt K., Berg C., Berg G. (2007b). The bryophyte genus *Sphagnum* is a reservoir for powerful and extraordinary antagonists and potentially facultative human pathogens. FEMS Microbiology Ecology 61: 38–53. https://doi.org/10.1111/j.1574-6941.2007.00323.x.

Oshkin I.Y., Beck D.A., Lamb A.E., Tchesnokova V., Benuska G., McTaggart T.L., Kalyuzhnaya M.G., Dedysh S.N., Lidstrom M.E., Chistoserdova L. (2014). Methane-fed microbial microcosms show differential community dynamics and pinpoint taxa involved in communal response. The ISME journal 9: 1119. https://doi.org/10.1038/ismej.2014.203.

Paavilainen E., Päivänen J. (1995). Peatland forestry: ecology and principles, Vol. 111. Springer Science & Business Media. https://doi.org/10.1007/978-3-662-03125-4.

Page S.E., Rieley J.O., Banks C..J (2011). Global and regional importance of the tropical peatland carbon pool. Global Change Biology 17: 798–818. https://doi.org/10.1111/j.1365-2486.2010.02279.x.

Pangala S.R., Enrich-Prast A., Basso L.S., Peixoto R.B., Bastviken D., Hornibrook E.R., Gatti L.V., Marotta H., Calazans L.S.B., Sakuragui C.M., Bastos W.R. (2017). Large emissions from floodplain trees close the Amazon methane budget. Nature 552: 230–234. https://doi.org/10.1038/nature24639.

Parmentier F.J.W., Van Huissteden J., Kip N., Op Den Camp H.J.M., Jetten M.S.M., Maximov T.C., Dolman A.J. (2011). The role of endophytic methane-oxidizing bacteria in submerged *Sphagnum* in determining methane emissions of Northeastern Siberian tundra. Biogeosciences 8: 1267–1278. https://doi.org/10.5194/bg-8-1267-2011.

Peltoniemi K., Laiho R., Juottonen H., Bodrossy L., Kell D.K., Minkkinen K., Mäkiranta P., Mehtätalo L., Penttilä T., Siljanen H.M., Tuittila E.-S. (2016). Responses of methanogenic and methanotrophic communities to warming in varying moisture regimes of two boreal fens. Soil Biology and Biochemistry 97: 144–156. https://doi.org/10.1016/j.soilbio.2016.03.007.

Pester M., Bittner N., Deevong P., Wagner M., Loy A. (2010). A “rare biosphere” microorganism contributes to sulfate reduction in a peatland. ISME Journal 4: 1591. https://doi.org/10.1038/ismej.2010.75.

Pol A,. Heijmans K., Harhangi H.R., Tedesco D., Jetten M.S.M., Op Den Camp H.J.M. (2007). Methanotrophy below pH 1 by a new Verrucomicrobia species. Nature 450: 874–878. https://doi.org/10.1038/nature06222.

Popp T.J., Chanton J.P., Whiting G.J., Grant N. (2000). Evaluation of methane oxidation in the rhizosphere of a *Carex* dominated fen in north central Alberta, Canada. Biogeochemistry 51: 259–281. https://doi.org/10.1023/A:1006452609284.

Pouliot R., Hugron S., Rochefort L. (2015). *Sphagnum* farming: A long-term study on producing peat moss biomass sustainably. Ecological Engineering 74: 135–147. https://doi.org/10.1016/j.ecoleng.2014.10.007.

Pratscher J., Vollmers J., Wiegand S., Dumont M.G., Kaster A.-K. (2018). Unravelling the identity, metabolic potential, and global biogeography of the atmospheric methane-oxidising Upland Soil Cluster α. Environmental Microbiology https://doi.org/10.1111/1462-2920.14036.

Prosser J.I. (2015). Dispersing misconceptions and identifying opportunities for the use of "omics" in soil microbial ecology. Nature Reviews Microbiology 13: 439–446. https://doi.org/10.1038/nrmicro3468.

Radajewski S., McDonald I.R., Murrell J.C. (2003). Stable-isotope probing of nucleic acids: A window to the function of uncultured microorganisms. Current Opinion in Biotechnology 14: 296–302. https://doi.org/10.1016/S0958-1669(03)00064-8.

Raghoebarsing A.A., Smolders A.J., Schmid M.C., Rijpstra W.I.C., Wolters-Arts M., Derksen J., Jetten M.S., Schouten S., Damsté J.S.S., Lamers L.P., Roelofs J.G. (2005). Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. Nature 436: 1153–1156. https://doi.org/10.1038/nature03802.

Raghoebarsing A.A., Pol A., Van de Pas-Schoonen K.T., Smolders A.J., Ettwig K.F., Rijpstra W.I.C., Schouten S., Damsté J.S.S., den Camp H.J.O., Jetten M.S., Strous M. (2006). A microbial consortium couples anaerobic methane oxidation to denitrification. Nature 440: 918–921. https://doi.org/10.1038/nature04617.

Reumer M., Harnisz M., Lee H.J., Reim A., Grunert O., Putkinen A., Fritze H., Bodelier P.L., Ho A. (2018). Impact of peat mining , and restoration on methane turnover potentials and methane-cycling microorganisms in a northern bog. Applied and Environmental Microbiology 84: e02218-17. https://doi.org/10.1128/AEM.02218-17.

Rochefort L., Quinty F., Campeau S., Johnson K., Malterer T. (2003). North American approach to the restoration of wetlands. Wetlands Ecology and Management 11: 3–20. https://doi.org/10.1023/A:1022011027946.

Rooney-Varga J.N., Giewat M.W., Duddleston K.N., Chanton J.P., Hines M.E. (2007). Links between archaeal community structure, vegetation type and methanogenic pathway in Alaskan peatlands. FEMS Microbiology Ecology 60: 240–251. https://doi.org/10.1111/j.1574-6941.2007.00278.x.

Rotaru A.E., Shrestha P.M., Liu F., Shrestha M., Shrestha D., Embree M., Zengler K., Wardman C., Nevin K.P., Lovley D.R. (2014). A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to *Methanosaeta* for the reduction of carbon dioxide to methane. Energy & Environmental Science 7: 408–415. https://doi.org/10.1039/C3EE42189A.

Roulet N.T., Moore T.R. (1995). The effect of forestry drainage practices on the emission of methane from northern peatlands. Canadian Journal of Forest Research 25: 491–499. https://doi.org/10.1139/x95-055.

Rydin H., Clymo R.S. (1989). Transport of carbon and phosphorus compounds about *Sphagnum*. Proceedings of the Royal Society B: Biological Sciences 237: 63–84. https://doi.org/10.1098/rspb.1989.0037.

Rydin H., Jeglum J.K. (2013). *Sphagnum* – the builder of boreal peatlands. In: The Biology of Peatlands. Oxford University Press. https://doi.org/10.1093/acprof:osobl/9780199602995.003.0004.

Saarnio S., Wittenmayer L., Merbach W. (2004). Rhizospheric exudation of *Eriophorum vaginatum* L. — Potential link to methanogenesis. Plant and Soil 267: 343–355. https://doi.org/10.1007/s11104-005-0140-3.

Salm J.O., Maddison M., Tammik S., Soosaar K., Truu J., Mander Ü. (2012). Emissions of $CO_2$, $CH_4$ and $N_2O$ from undisturbed, drained and mined peatlands in Estonia. Hydrobiologia 692: 41–55. https://doi.org/10.1007/s10750-011-0934-7.

Schink B. (1997). Energetics of syntrophic cooperation in methanogenic degradation. Microbiology and molecular biology reviews 61: 262–280.

Schmidt O., Hink L., Horn M.A., Drake H.L. (2016). Peat: home to novel syntrophic species that feed acetate- and hydrogen-scavenging methanogens. The ISME Journal 10: 1954. https://doi.org/10.1038/ismej.2015.256.

Schrier-Uijl A.P., Kroon P.S., Leffelaar P.A., van Huissteden J.C., Berendse F., Veenendaal E.M. (2010). Methane emissions in two drained peat agro-ecosystems with high and low agricultural intensity. Plant and Soil 329: 509–520. https://doi.org/10.1007/s11104-009-0180-1.

Schuur E.A.G., McGuire A.D., Schädel C., Grosse G., Harden J.W., Hayes D.J., Hugelius G., Koven C.D., Kuhry P., Lawrence D.M., Natali S.M. (2015). Climate change and the permafrost carbon feedback. Nature 520: 171–179. https://doi.org/10.1038/nature14338.

Sharp C.E., Smirnova A.V., Graham J.M., Stott M.B., Khadka R., Moore T.R., Grasby S.E., Strack M., Dunfield P.F. (2014). Distribution and diversity of Verrucomicrobia methanotrophs in geothermal and acidic environments. Environmental microbiology 16: 1867–1878. https://doi.org/10.1111/1462-2920.12454.

Shaw A.J., Devos N., Cox C.J., Boles S.B., Shaw B., Buchanan A.M., Cave L., Seppelt R. (2010). Peatmoss (*Sphagnum*) diversification associated with Miocene Northern Hemisphere climatic cooling? Molecular Phylogenetics and Evolution 55: 1139–1145. https://doi.org/10.1016/j.ympev.2010.01.020.

Shcherbakov A.V., Bragina A.V., Kuzmina E.Y., Berg C., Muntyan A.N., Makarova N.M., Malfanova N.V., Cardinale M., Berg G., Chebotar V.K., Tikhonovich I.A., (2013). Endophytic bacteria of *Sphagnum* mosses as promising objects of agricultural microbiology. Microbiology 82: 306–315. https://doi.org/10.1134/S0026261713030107.

Shi Y., Wang Z., He C., Zhang X., Sheng L., Ren X. (2017). Using $^{13}C$ isotopes to explore denitrification-dependent anaerobic methane oxidation in a paddy-peatland. Scientific Reports 7: 40848. https://doi.org/10.1038/srep40848.

Singer D.K., Jackson S.T., Madsen B.J., Wilcox D.A. (1996). Differentiating climatic and successional influences on long-term development of a marsh. Ecology 77: 1765–1778. https://doi.org/10.2307/2265782.

Soudzilovskaia N.A., Cornelissen J.H.C., During H.J., Van Logtestun R.S.P., Lang S.I., Aerts R. (2010). Similar cation exchange capacities among bryophyte species refute a presumed mechanism of peatland acidification. Ecology 91: 2716–2726. https://doi.org/10.1890/09-2095.1.

Steinberg L.M., Regan J.M. (2008). Phylogenetic comparison of the methanogenic communities from an acidic, oligotrophic fen and an anaerobic digester treating municipal wastewater sludge. Applied and Environmental Microbiology 74: 6663–6671. https://doi.org/10.1128/AEM.00553-08.

Stoecker K., Bendinger B., Schöning B., Nielsen P.H., Nielsen J.L., Baranyi C., Toenshoff E.R., Daims H., Wagner M. (2006). Cohn's *Crenothrix* is a filamentous methane oxidizer with an unusual methane monooxygenase. Proceedings of the National Academy of Sciences 103: 2363–2367. https://doi.org/10.1073/pnas.0506361103.

Strack M., Mwakanyamale K., Hassanpour Fard G., Bird M., Bérubé V., Rochefort L. (2017). Effect of plant functional type on methane dynamics in a restored minerotrophic peatland. Plant and Soil 410: 231–246. https://doi.org/10.1007/s11104-016-2999-6.

Stralis-Pavese N., Sessitsch A., Weilharter A., Reichenauer T., Riesing J., Csontos J., Murrell J.C., Bodrossy L. (2004). Optimization of diagnostic microarray for application in analysing landfill

methanotroph communities under different plant covers. Environmental Microbiology 6: 347–363. https://doi.org/10.1111/j.1462-2920.2004.00582.x.

Stralis-Pavese N., Abell G.C.J., Sessitsch A., Bodrossy L. (2011). Analysis of methanotroph community structure using a *pmoA*-based microarray. Nature Protocols 6: 609–624. https://doi.org/10.1038/nprot.2010.191.

Ström L., Ekberg A., Mastepanov M., Christensen T.R. (2003). The Effect of vascular plants on carbon turnover and methane emissions from a tundra wetland. Global Change Biology 9: 1185–1192. https://doi.org/10.1046/j.1365-2486.2003.00655.x.

Sundh I., Nilsson M., Granberg G., Svensson B.H. (1994). Depth distribution of microbial production and oxidation of methane in northern boreal peatlands. Microbial Ecology 27: 253–265. https://doi.org/10.1007/BF00182409.

Sundh I., Mikkelä C., Nilsson M., Svensson B.H. (1995). Potential aerobic methane oxidation in a *Sphagnum*-dominated peatland - controlling factors and relation to methane emission. Soil Biology and Biochemistry 27: 829–837. https://doi.org/10.1016/0038-0717(94)00222-M.

Tahvanainen T. (2011). Abrupt ombrotrophication of a boreal aapa mire triggered by hydrological disturbance in the catchment. Journal of Ecology 99: 404–415. https://doi.org/10.1111/j.1365-2745.2010.01778.x.

Tarvainen O., Laine A.M., Peltonen M., Tolvanen A. (2013). Mineralization and decomposition rates in restored pine fens. Restoration Ecology 21: 592–599. https://doi.org/10.1111/j.1526-100X.2012.00930.x.

van Teeseling M.C.F., Pol A., Harhangi H.R., van der Zwart S., Jetten M.S.M., Op den Camp H.J.M., van Niftrik L. (2014). Expanding the verrucomicrobial methanotrophic world: Description of three novel species of *Methylacidimicrobium* gen. nov. Applied and Environmental Microbiology 80: 6782–6791. https://doi.org/10.1128/AEM.01838-14.

Theisen A.R., Ali M.H., Radajewski S., Dumont M.G., Dunfield P.F., McDonald I.R., Dedysh S.N., Miguez C.B., Murrell J.C., (2005). Regulation of methane oxidation in the facultative methanotroph *Methylocella silvestris* BL2. Molecular Microbiology 58: 682–692. https://doi.org/10.1111/j.1365-2958.2005.04861.x.

Thormann M.N., Szumigalski A.R., Bayley S.E. (1999). Aboveground peat and carbon accumulation potentials along a bog-fen-marsh wetland gradient in southern boreal Alberta, Canada. Wetlands 19: 305–317. https://doi.org/10.1007/BF03161761.

Tuittila E.-S., Komulainen V.M., Vasander H., Nykänen H., Martikainen P.J., Laine J. (2000a). Methane dynamics of a restored cut-away peatland. Global Change Biology 6: 569–581. https://doi.org/10.1046/j.1365-2486.2000.00341.x.

Tuittila E.-S., Vasander H., Laine J. (2000b). Impact of rewetting on the vegetation of a cut-away peatland. Applied Vegetation Science 3: 205–212. https://doi.org/10.2307/1478999.

Tuittila E.-S., Vasander H., Laine J. (2004). Sensitivity of C sequestration in reintroduced *Sphagnum* to water-level variation in a cutaway peatland. Restoration Ecology 12: 483–493. https://doi.org/10.1111/j.1061-2971.2004.00280.x.

Tuittila E.-S., Väliranta M., Laine J., Korhola A. (2007). Quantifying patterns and controls of mire vegetation succession in a southern boreal bog in Finland using partial ordinations. Journal of Vegetation Science 18: 891–902. https://doi.org/10.1111/j.1654-1103.2007.tb02605.x.

Tuittila E.-S., Juutinen S., Frolking S., Väliranta M., Laine A.M., Miettinen A., Seväkivi M.L., Quillet A., Merilä P. (2013). Wetland chronosequence as a model of peatland development: Vegetation succession, peat and carbon accumulation. Holocene 23: 25–35. https://doi.org/10.1177/0959683612450197.

Turetsky M.R. (2003). The role of bryophytes in carbon and nitrogen cycling. The Bryologist 106: 395–409. https://doi.org/10.1639/05.

Turetsky M.R., Kotowska A., Bubier J., Dise N.B., Crill P., Hornibrook E.R., Minkkinen K., Moore T.R., Myers-Smith I.H., Nykänen H., Olefeldt D. (2014). A synthesis of methane emissions from 71 northern, temperate, and subtropical wetlands. Global Change Biology 20: 2183–2197. https://doi.org/10.1111/gcb.12580.

Urbanová Z., Picek T., Bárta J. (2011). Effect of peat re-wetting on carbon and nutrient fluxes, greenhouse gas production and diversity of methanogenic archaeal community. Ecological Engineering 37: 1017–1026. https://doi.org/10.1016/j.ecoleng.2010.07.012.

Vanwonterghem I., Evans P.N., Parks D.H., Jensen P.D., Woodcroft B.J., Hugenholtz P., Tyson G.W. (2016). Methylotrophic methanogenesis discovered in the archaeal phylum Verstraetearchaeota. Nature microbiology 1: 16170. https://doi.org/10.1038/nmicrobiol.2016.170.

Vasander H., Tuittila E.-S., Lode E., Lundin L., Ilomets M., Sallantaus T., Heikkilä R., Pitkänen M.L., Laine J. (2003). Status and restoration of peatlands in northern Europe. Wetlands Ecology and Management 11: 51–63. https://doi.org/10.1023/A:1022061622602.

Vasil'eva L.V., Berestovskaya Y.Y., Zavarzin G.A. (1999). Psychrophilic acidophilic methanotrophs from the *Sphagnum* zone of permafrost. Doklady Akademii Nauk 368: 125–128.

Vigliotta G., Nutricati E., Carata E., Tredici S.M., De Stefano M., Pontieri P., Massardo D.R., Prati M.V., De Bellis L., Alifano P. (2007). *Clonothrix fusca* Roze 1896, a filamentous, sheathed, methanotrophic γ-proteobacterium. Applied and Environmental Microbiology 73: 3556–3565. https://doi.org/10.1128/AEM.02678-06.

Vile M.A., Wieder R.K., Živković T., Scott K.D., Vitt D.H., Hartsock J.A., Iosue C.L., Quinn J.C., Petix M., Fillingim H.M., Popma J.M. (2014). $N_2$-fixation by methanotrophs sustains carbon and nitrogen accumulation in pristine peatlands. Biogeochemistry 121: 317–328. https://doi.org/10.1007/s10533-014-0019-6.

Vorobev A.V., Baani M., Doronina N.V., Brady A.L., Liesack W., Dunfield P.F., Dedysh S.N. (2011). *Methyloferula stellata* gen. nov., sp. nov., an acidophilic, obligately methanotrophic bacterium that possesses only a soluble methane monooxygenase. International Journal of Systematic and Evolutionary Microbiology 61: 2456–2463. https://doi.org/10.1099/ijs.0.028118-0.

Väliranta M., Salojärvi N., Vuorsalo A., Juutinen S., Korhola A., Luoto M., Tuittila E.-S. (2017). Holocene fen – bog transitions , current status in Finland and future perspectives. The Holocene 27: 752-764. https://doi.org/10.1177/0959683616670471.

Waddington J.M., Warner K.D., Kennedy G.W. (2002). Cutover peatlands: A persistent source of atmospheric $CO_2$. Global Biogeochemical Cycles 16: 1–7. https://doi.org/10.1029/2001GB001398.

Waddington J.M., Day S.M. (2007). Methane emissions from a peatland following restoration. Journal of Geophysical Research: Biogeosciences 112: 1–11. https://doi.org/10.1029/2007JG000400.

Wagner R., Zona D., Oechel W., Lipson D. (2017). Microbial community structure and soil ph correspond to methane production in arctic Alaska soils. Environmental microbiology 19: 3398–3410. https://doi.org/10.1111/1462-2920.13854.

Warren M.J., Lin X., Gaby J.C., Kretz C.B., Kolton M., Morton P.L., Pett-Ridge J., Weston D.J., Schadt C.W., Kostka J.E., Glass J.B. (2017). Molybdenum-based diazotrophy in a *Sphagnum* peatland in northern Minnesota. Applied and Environmental Microbiology 83: e01174-17. https://doi.org/10.1128/AEM.01174-17.

Wartiainen I., Hestnes A.G., McDonald I.R., Svenning M.M. (2006). *Methylocystis rosea* sp. nov., a novel methanotrophic bacterium from Arctic wetland soil, Svalbard, Norway (78° N). International Journal of Systematic and Evolutionary Microbiology 56: 541–547. https://doi.org/10.1099/ijs.0.63912-0.

Weber H.S., Habicht K.S., Thamdrup B. (2017). Anaerobic methanotrophic archaea of the ANME-2d cluster are active in a low-sulfate, iron-rich freshwater sediment. Frontiers in Microbiology 8: 619. https://doi.org/10.3389/fmicb.2017.00619.

Whalen S.C., Reeburgh W.S. (2000). Methane oxidation, production, and emission at contrasting sites in a boreal bog. Geomicrobiology Journal 451: 237–251. https://doi.org/10.1080/01490450050121198.

Whalen S.C. (2005). Natural wetlands and the atmosphere. Environmental Engineering Science 22: 73–94. https://doi.org/10.1089/ees.2005.22.73.

Whittenbury R., Phillips K.C.C., Wilkinson J.F. (1970). Enrichment, isolation and some properties of methane-utilizing bacteria. Journal of general microbiology 61: 205–18. https://doi.org/10.1099/00221287-61-2-205.

Wilson D., Blain D., Couwenberg J., Evans C.D., Murdiyarso D., Page S.E., Renou-Wilson F., Rieley J.O., Sirin A., Strack M, Tuittila E.-S. (2016). Greenhouse gas emission factors associated with rewetting of organic soils. Mires and Peat 17: 1–28.

van Winden J.F., Kip N., Reichart G.J., Jetten M.S.M., Op den Camp H.J.M., Damsté J.S.S. (2010). Lipids of symbiotic methane-oxidizing bacteria in peat moss studied using stable carbon isotopic labelling. Organic Geochemistry 41: 1040–1044. https://doi.org/10.1016/j.orggeochem.2010.04.015.

van Winden J.F., Talbot H.M., Kip N., Reichart G.J., Pol A., McNamara N.P., Jetten M.S., Op den Camp H.J.M., Damste J.S.S. (2012). Bacteriohopanepolyol signatures as markers for methanotrophic bacteria in peat moss. Geochimica et Cosmochimica Acta 77: 52–61. https://doi.org/10.1016/j.gca.2011.10.026.

Yachi S., Loreau M. (1999). Biodiversity and ecosystem productivity in a fluctuating environment : The insurance hypothesis. Proceedings of the National Academy of Sciences 96: 1463–1468. https://doi.org/10.1073/pnas.96.4.1463.

Yimga M.T., Dunfield P.F., Ricke P., Liesack W. (2003). Wide distribution of a novel *pmoA*-like gene copy among type II methanotrophs, and its expression in *Methylocystis* strain SC2. Applied and environmental microbiology 69: 5593–5602. https://doi.org/10.1128/AEM.69.9.5593-5602.2003.

Yrjälä K., Tuomivirta T., Juottonen H., Putkinen A., Lappi K., Tuittila E.-S., 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. https://doi.org/10.1111/j.1365-2486.2010.02290.x.

Yu Z.C. (2012). Northern peatland carbon stocks and dynamics: A review. Biogeosciences 9: 4071–4085. https://doi.org/10.5194/bg-9-4071-2012.

Zheng Y., Harris D.F., Yu Z., Fu Y., Poudel S., Ledbetter R.N., Fixen K.R., Yang Z.Y., Boyd E.S., Lidstrom M.E., Seefeldt L.C. (2018) A pathway for biological methane production using bacterial iron-only nitrogenase. Nature microbiology 3: 281–286. https://doi.org/10.1038/s41564-017-0091-5