Microbial Communities in Boreal Peatlands

Responses to Climate Change and
Nitrogen and Sulfur Depositions

Magalí Martí Generó

Linköping Studies in Arts and Science No. 715
Faculty of Arts and Sciences
Linköping 2017

Linköping Studies in Arts and Science ● No. 715

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Distributed by:
Department of Thematic Studies – Environmental Change
Linköping University
581 83 Linköping

Magalí Martí Generó
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Edition 1:1
ISBN 978-91-7685-533-1
ISSN 0282-9800

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Department of Thematic Studies – Environmental Change 2017

Cover image: Photo by Magalí Martí Generó (Männijärve Bog, Estonia)
Printed by: LiU-Tryck, Linköping 2017

*Al meu avi de l'Arumi, Jaume
Amb qui vaig descobrir i aprendre a estimar la natura*

*Al meu avi de Vic, Joan
Qui m'ha ensenyat que el saber no ocupa lloc*

Abstract

Peatlands play a substantial role in regulating the global carbon balance and concentrations of the greenhouse gases CO₂ and CH₄ in the atmosphere, and are thus of utmost importance from a climate change perspective. Any changes of peatland functions due to natural or anthropogenic perturbations may result in changes in these ecosystem services. Soil microbial communities are essential drivers of biogeochemical processes, including the carbon cycle. In order to fully understand the effect of environmental perturbations on peatland functions, it is essential to understand how microbial communities are affected. The aim of the research presented in this thesis was to investigate the responses of the peat microbial communities to climate change and increased precipitation of nitrogen (N) and sulfur (S) compounds. High-throughput sequencing approaches were used to investigate the taxonomic and functional composition of microbial communities, and quantitative PCR was used to specifically target the methanogen community. Two field studies including three ombrotrophic peatlands each that differed in climatological conditions and atmospheric N and S depositions, were used to investigate and compare the effect of large- and local-scale environmental conditions on microbial communities. The results show that the variation in geo-climatological (temperature and precipitation) and atmospheric deposition conditions along the latitudinal gradient modulate the peat microbial community composition and the abundance of active methanogens to a greater extent than site-related microhabitats. Furthermore, a tight coupling between the plant community composition of a site and the composition of its microbial community was observed, and was found to be mainly driven by plants rather than microorganisms. These co-occurrence networks are strongly affected by seasonal climate variability and the interactions between species in colder areas are more sensitive to climate change. The long-term effects of warming and increased N and S depositions on the peat microbial communities were further investigated using an 18-year in-situ peatland experiment simulating these perturbations. The impacts of each of these perturbations on the microbial community were found to either multiply or counteract one another, with enhanced N deposition being the most important factor. While the long-term perturbations resulted in a substantial shift in the taxonomic composition of microbial communities, only minor changes occurred in genome-encoded functional traits, indicating a functional redundancy. This could act as a buffer maintaining ecosystem functioning when challenged by multiple stressors, and could limit future changes in greenhouse gases and carbon exchange.

Keywords: Microbial communities, methanogens, plant communities, peatland, temperature, nitrogen, long-term, field experiment, high-throughput sequencing.

Sammanfattning

Myrmarker har en stor roll i regleringen av den globala kolbalansen och koncentrationerna av koldioxid och metan i atmosfären, vilket gör dem till speciellt viktiga ekosystem ur ett klimatförändringsperspektiv. Förändringar av myrmarker genom naturlig utveckling eller antropogen påverkan kan därför få långtgående störningar av myrars klimatreglerande funktion. Mikroorganismer har en avgörande roll i biogeokemiska processer genom att de bryta ned organisk material i mark och därmed styra kretsloppet. För att förstå hur myrsystemen reagerar på störningar är det därför väsentligt att veta hur mikroorganismssamhällena reagerar genom förändringar i sammansättning och biogeokemisk aktivitet. Målet för studierna, som ligger till grund för denna avhandling, var att undersöka hur mikroorganismssamhällen i myrmarker reagerar på uppvärmning genom klimatförändring och ökade kväve- (N) och svavel- (S) halter i nederbörd. High through-put sekvensering användes för att studera taxonomiska och funktionella egenskaper hos mikroorganismerna i myrmarker och kvantitativ PCR användes för att mer specifikt studera de metanbildande arkeerna. Två fältkampanjer vardera omfattande tre ombrotrofa myrmarker med olika klimatförhållanden och olika mängder N och S i nederbörden användes för att undersöka lokala och storskaliga effekter på myrarnas mikrobiella samhällen. Resultaten visade att latitudinell variation i geoklimatologiska förhållanden (temperatur och nederbördsmängd) och deposition av näringsämnen hade en påverkan på sammansättningen av de mikrobiella samhällena och aktiva metanbildare före variationen i den kemiska miljön inom varje specifik myr. Myrväxtsamhällenas sammansättning för en specifik myr visades sig i stor utsträckning styra sammansättningen av motsvarande mikrobiella samhällen i torvprofilen. Detta framgick klart av en analys av samexisterande nätverk av mikroorganismssamhällen och motsvarande växtsamhällen i en studie av tre geografiskt skilda myrmarker med olika kvävedeposition. Effekterna av klimatförändring och nederbörd med olika mängder av N och S studerades mer specifikt genom att analysera de mikrobiella samhällena i ett långliggande (18 år) försök. Påverkan av var och en av dessa manipulationer antingen förstärktes eller minskades, när de förekom i kombinationer. Ökad kvävedeposition var den faktor som hade starkast effekt. De långvariga störningarna medförde stora förändringar i den mikrobiella taxonomin inom samhällena. Detta återspeglades dock inte i den fysiologiska kapaciteten, vilket visar att det finns en stark buffring i myrarnas mikrobiella funktion. Detta tyder på att framtida utveckling av myrmarker i relation till olika störningar sannolikt inte kommer att påverka myrarnas roll för kolbalans och växthusgasutbyte med atmosfären.

Nyckelord: Mikrobiella samhällen, metanogener, växtsamhällen, myrmarker, torv, temperatur, kväve, långtid, fältexperiment, high-throughput-sekvensering.

Table of Contents

- List of papers i
- Author’s contributions..... ii
- Abbreviations iii
- 1. Introduction..... 1
- 2. Research objectives 5
- 3. Background..... 7
 - 3.1. The boreal peatland ecosystem 7
 - 3.2. Microbial degradation of organic matter in peatlands..... 9
 - 3.3. Interactions between plant and microbial communities in peatlands 12
 - 3.4. Molecular detection and characterisation of microbial communities..... 13
- 4. Methods 17
 - 4.1. Site selection and sample collection 17
 - 4.1.1. Ombrotrophic raised bogs (Papers I, II) 17
 - 4.1.2. Field experiment: Degerö Stormyr (Papers III, IV) 18
 - 4.2. Molecular methods 20
 - 4.3. Statistical analysis 22
 - 4.4 Co-occurrence network 23
- 5. Results and discussion 25
 - 5.1. Local- and large-scale variability of microbial communities (Papers I, II)..... 25
 - 5.1.1. Spatial variability of Archaea and Bacteria community compositions 26
 - 5.1.2. Spatial variability of methanogenic archaeal community 27
 - 5.2. Effects of warming and increased atmospheric N and S depositions on microbial communities (Papers III, IV) 29
 - 5.2.1. Microbial community responses to warming and increased N and S depositions (Paper III) .. 29
 - 5.2.2. Responses of methanogens to warming and increased N and S depositions (Paper IV) .. 33
 - 5.2.3. Effects of interactions between simultaneous warming and increased N and S depositions (Papers III, IV) 35
 - 5.3. Plant-microorganism interactions (Papers I, III, IV) 36
 - 5.4. Summary of the results 37
- 6. Outcomes and reflections 41
- Acknowledgements 45
- References..... 47

List of papers

The thesis is based on the following papers, referred to in the text by the corresponding Roman numerals:

- I. **Magalí Martí**, Vincent E.J. Jassey, Bjorn J.M. Robroek, Bo H. Svensson. Interactions between microbial and plant communities have disproportional responses to projected climate change in boreal peatlands. *Manuscript*.
- II. **Magalí Martí**, Heli Juottonen, Bjorn J.M. Robroek, Kim Yrjälä, Åsa Danielsson, Per-Eric Lindgren, Bo H. Svensson. (2015). Nitrogen and methanogen community composition within and among three *Sphagnum* dominated peatlands in Scandinavia. *Soil Biology & Biochemistry* 81: 204-211.
- III. **Magalí Martí**, Alexander Eiler, Moritz Buck, Stefan Bertilsson, Waleed Abu Al-Soud, Sören Sörensen, Mats B. Nilsson, Bo H. Svensson. Large changes in prokaryote community composition after 18-years of simulated interactions of warming and increased nitrogen and sulfur depositions in a peatland experiment. *In review*.
- IV. **Magalí Martí**, Mats B. Nilsson, Åsa Danielsson, Per-Eric Lindgren, Bo H. Svensson. Strong positive interaction effects on the abundance of active methanogens in a boreal oligotrophic mire as a response of a long-term experimental increase in temperature and nitrogen deposition. *In review*

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Author's contributions

- I. The author, together with BJMR, conducted the sample collection process. The author, together with VEJJ, conducted the data analysis and wrote the first draft of the results section of the manuscript. The author coordinated the writing process and performed the laboratory analysis of the peat samples.
- II. The author conducted the laboratory analysis of the peat samples, data analyses, wrote the first draft of the manuscript, and coordinated the writing process.
- III. The author, together with MBN and BHS, conducted the sample collection process. The author, together with AE, conducted the data analysis and wrote the first draft of the results section of the manuscript. The author coordinated the writing process and performed the laboratory analysis.
- IV. The author, together with MBN and BHS, conducted the sample collection process. The author conducted the data analysis, wrote the first draft of the manuscript, and coordinated the writing process.

Abbreviations

AWTL	Above the water table level
BWTL	Below the water table level
C	Carbon
CO ₂	Carbon dioxide
CH ₄	Methane
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
FWTL	Flanking the water table level
GHGs	Greenhouse gases
H ₂	Hydrogen
MCR	Methyl-coenzyme M reductase (enzyme)
<i>mcrA</i>	Gene encoding methyl-coenzyme M reductase I α -subunit
N	Nitrogen
NGS	Next-generation sequencing
NMDS	Non-metric multidimensional scaling
OTU	Operational taxonomic unit
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
S	Sulfur
SAOB	Syntrophic acetate-oxidising bacteria
SRB	Sulfate-reducing bacteria
T-RFLP	Terminal restriction fragment length polymorphism
WTL	Water table level

1. Introduction

Microbial communities are ubiquitous, and exhibit vast phylogenetic and physiological diversity. For example, one gram of soil has been estimated to contain up to 10^7 species (Torsvik et al., 2002; Gans et al., 2005). These communities are essential drivers of biogeochemical processes that directly affect ecosystem services and functions (e.g. Falkowski et al., 2008; Rousk and Bengtson, 2014; Graham et al., 2016). As such, the abundance and diversity of, as well as interactions between, different functional groups (microbial community structure) play key roles in controlling the dynamics of carbon (C) and other nutrients within ecosystems. For example, the abundance of specific microbial taxa that are able to produce methane is associated with the rate of methane emission in permafrost ecosystems (McCalley et al., 2014). The resistance and resilience of interactions between species (ecological networks), with regard to environmental perturbations, are essential for ecosystem stability and are dependent on the microbial community structure (reviewed in Griffiths and Philippot, 2013). For instance, the peat microbial community has been reported to be highly resilient after 8 years of increased sulfate deposition and resistant or resilient to 11 years of simulated warming (Strickman et al., 2016; Weedon et al., 2017). Microbial communities change their structures in response to climate change and environmental perturbations (e.g. warming, altered precipitation, increased carbon dioxide (CO₂), increased atmospheric nitrogen (N) and sulphur (S) depositions), and the extent to which this occurs is dependent on the ecosystem type (e.g. Castro et al., 2010; Fierer et al., 2012; Hu et al., 2013; Shen et al., 2014; Contosta et al., 2015; Strickman et al., 2016; Zeng et al., 2016). These changes in microbial community structure may affect ecosystem functioning and stability, which in turn may impact the climate system via changes in greenhouse gas (GHG) turnover and other ecosystem-scale processes. The impact of environmental perturbations on ecosystems may be attenuated to a certain extent by functional redundancy, assuming that some species can mediate similar functions in the ecosystem (Lawton and Brown, 1993).

All ecosystems are affected by climate change and other perturbations caused by anthropogenic activities, which can impact essential ecosystem services. Warming leads to an increase in microbial biomass and metabolic activity, and to a shift in composition which may result in increased rates of organic matter decomposition and consequent higher turnover and potential loss of soil organic C (e.g. Castro et al., 2010; Luo and Weng, 2011; Shen et al., 2014). The global average surface temperature has increased by an average of 0.85°C between 1880 and 2012, and is

projected to have increased by an estimated 1.1 to 6.4°C by the end of the twenty-first century. At northern latitudes, an even greater degree of warming has been projected (IPCC, 2013). The availability of reactive N in the atmosphere has increased due to industrial and agricultural activities, and is projected to continue to increase in line with global population growth (Galloway et al., 2003; Galloway et al., 2004). Consequently, annual N deposition rates in terrestrial ecosystems are estimated to increase by a factor of approximately 2.5 (Lamarque et al., 2005). Combustion processes also result in sulfur dioxide emissions to the atmosphere. S emission peaked in the 1970s but, following a period of decline, an increase in emission rates has resumed (Gauci et al., 2004; Smith et al., 2011). Atmospheric N and S depositions may lead to eutrophication, acidification, biodiversity changes in ecosystems, and increased rates of litter decomposition. The extent of shifts in microbial composition, biomass and diversity, and rates of decomposition are dependent on both ecosystem type and time-scale (e.g. Fierer et al., 2012; Hu et al., 2013; Contosta et al., 2015; Strickman et al., 2016; Zeng et al., 2016; Xu et al., 2017). In peatlands, for example, microbial decomposition rates have been found to increase with N loading, resulting in C loss (Bragazza et al., 2006; Currey et al., 2010).

As northern peatlands (latitude 40° to 70°N) generally exist in areas of low temperatures and are nutrient-poor, they are among the most vulnerable ecosystems to projected climate change and increases in atmospheric N and S deposition (Limpens et al., 2008; Artz, 2009; Luo and Weng, 2011). One of the most important services provided by ecosystems, and peatlands in particular, is the regulation and stabilisation of the climate, as their C cycle dynamics (mediated by plants and soil microorganisms) control the exchange of GHGs – primarily CO₂, methane (CH₄), and nitrous oxide (N₂O) – with the atmosphere. Since the last de-glaciation, undisturbed peatland ecosystems have contributed to global cooling by generally functioning as net sinks of atmospheric CO₂ due to their slow rates of organic matter decomposition (Frolking and Roulet, 2007; Roulet et al., 2007; Nilsson et al., 2008; Turetsky et al., 2014). Northern peatlands are particularly important; covering approximately 3% of the land area of the Earth, they contain roughly 30% of the global pool of stored soil C and 10% of available freshwater (Gorham, 1991; Yu, 2012). However, peatlands also contribute to warming, as they are a major source of atmospheric CH₄ as a consequence of their water-saturated conditions, which foster anaerobic decomposition of the peat organic matter (e.g. Roulet et al., 2007; Nilsson et al., 2008; Turetsky et al., 2014). The estimated CH₄ emissions of northern peatlands make up approximately 11% of global emissions (Wuebbles and Hayhoe, 2002). Methane is more efficient at absorbing radiation than CO₂; thus, over a one hundred-year period, the impact of CH₄ on climate change is estimated to be 25 times greater than

that of CO₂ (Forster et al., 2007). The balance between the CO₂ withdrawal (cooling effect) and CH₄ emissions (warming effect) of peatlands has until the present resulted in a negative net radiative force, i.e. a global cooling effect. It is thus essential to understand how peatlands are affected by environmental and climatic factors in order to better predict their responses to climate change (Granberg et al., 2001; Galloway et al., 2004; Gauci et al., 2004; Phoenix et al., 2012; IPCC, 2013).

The effects of warming and nutrient deposition in northern peatlands on vegetation and GHG dynamics have been extensively studied (e.g. Gauci and Dise, 2002; Vile et al., 2003; Gauci et al., 2004; Bubier et al., 2007; Eriksson et al., 2010a, 2010b; Bragazza et al., 2012). However, microbial communities and their interactions with plant communities, that mediate the C turnover and nutrient mineralisation and uptake, have been less addressed. Therefore, to fully understand the effects of climate change and other perturbations on peatlands, it is essential to study how microbial communities are affected and respond. It has recently been demonstrated that predictive models for global biogeochemical cycles are improved when taking into account microbial community structures, particularly when including functional genes (Powell et al., 2015; Graham et al., 2016). With regard to peatlands, it has also been established that new insights into biogeochemical dynamics can be gained by studying peat microbial community structure and function, which can lead to further improvements to predict the responses of peatland ecosystems to global change (Bragazza et al., 2015).

2. Research objectives

Given the vulnerability of northern peatlands to a changing climate and their critical function as global climate regulators, there is a need to understand how these ecosystems respond to environmental change. The dynamics of microbial communities and their interactions with plant communities play a key role in mediating biogeochemical process related to climate regulation; thus, understanding the response of microbial communities to environmental perturbations will provide important insights into ecosystem sensitivity and resilience to climate and environmental change. Although there exists a substantial body of literature on C balance dynamics and concomitant methane emissions from northern peatlands in relation to plant distribution, warming, water table fluctuations, and the deposition of anthropogenic-derived pollutants, there is a lack of knowledge regarding microbial community dynamics and interactions with plants.

The overall aim of the research presented in this thesis was to investigate how microbial communities in boreal peatlands respond to environmental change. In order to address this, three specific research objectives were developed. **Firstly**, the impact of local- and large-scale environmental conditions on peat microbial communities were assessed and compared (Papers I, II). **Secondly**, based on the outcomes of the first research objective, three environmental perturbations (warming and increased rates of N and S deposition) were selected and analysed in order to understand their effect on microbial communities (Papers III, IV). **Finally**, the relationship between plant and microbial communities in relation to environmental perturbations was further examined (Paper I).

These research objectives were approached by a combination of two field studies of peatlands with naturally differing climatological and nutrient conditions, and a long-term in-situ experiment simulating warming and N and S deposition (Table 1). Two of the studies investigated the taxonomic and functional composition of microbial communities, and two focused specifically on methanogenic archaeal community. These studies, as presented in the four articles that form the basis of the research presented in this thesis, are summarised in Table 1.

Table 1. An overview of the contributions of each paper to the research presented in this thesis, showing the microorganisms studied and the traits targeted, as well as the study sites and environmental perturbations that were investigated. T: Temperature. N: Nitrogen. S: Sulfur. P: Precipitation.

Paper	Microorganisms	Trait	Study site	Environmental perturbation
I	Prokaryotic community	Taxonomic composition	Three northern peatlands	T, N, S, P
II	Methanogenic archaea	<i>mcrA</i> gene expression	Three northern peatlands	T, N
III	Prokaryotic community	Taxonomic composition Functional potential	in-situ manipulations	T, N, S
IV	Methanogenic archaea	<i>mcrA</i> gene expression	in-situ manipulations	T, N, S

To provide a foundation for the outcomes and reflections of the research presented in this thesis, a background (Chapter 3) is presented, which briefly describes the boreal peatland ecosystem and gives an overview of the degradation of organic matter as mediated by the microbial community and the importance of the relationship between plants and microorganisms. The importance and implications of the molecular tools that were used to study microbial communities are also described in brief. In Chapter 4, the methodology used is presented and discussed in relation to site selection, sampling strategies and molecular and statistical approaches. Comprehensive descriptions of the methods are given in the four papers appended to this thesis. In Chapter 5, the results are presented and discussed according to the research aims outlined above. The final outcomes and reflections are presented in Chapter 6.

3. Background

3.1. The boreal peatland ecosystem

Peatlands are a type of wetland that are characterised by their unique ability to accumulate and store partially decomposed organic matter, referred to as 'peat' (Gorham, 1991). Peat formation (C storage) occurs due to an imbalance between the rates of primary production of plants and microbial decomposition. Current global C stock estimates for peatlands are 270 to 600 Pg of organic C (Gorham, 1991; Turunen et al., 2002; Yu, 2012). Decomposition occurs at a relatively slow rates due to a combination of several factors that energetically constrain microbial metabolism: water-saturated conditions and subsequent anoxia, recalcitrant *Sphagnum* moss tissue, relatively low peat temperature, and low pH (Frolking et al., 2001). Thus, peatlands are long-term C-storage ecosystems, and one of the most important ecosystem types with regard to the regulation of the global C cycle. The majority of peatlands are located at northern latitudes, with one-fifth present in the tropics (Holden, 2005).

There is a continuous hydrological gradient between two types of peatland: ombrotrophic bogs and minerotrophic fens (Rydin and Jeglum, 2006). Ombrotrophic bogs only receive water and nutrients from precipitation and are nutrient-poor, acidic ($\text{pH} < 4$), and *Sphagnum*-dominated. Minerotrophic fens, which also receive water and nutrients from groundwater, are richer in nutrients, have higher pH, and are dominated by vascular plants (mainly graminoids such as *Carex spp.*). The studies conducted for this thesis focused on ombrotrophic bogs. The vegetation in northern peatlands consists largely of *Sphagnum* mosses, while vascular plants are represented in the form of graminoids and dwarf shrubs. *Sphagnum* mosses are very efficient in retaining N and other nutrients, which is why, under natural conditions of low N availability, the vegetation community is dominated by *Sphagnum* (Clymo, 1963; Van Breemen, 1995). *Sphagnum* mosses also contain considerable amounts of uronic acids and polyphenols, which are strong inhibitors of microbial decomposition (Clymo and Hayward, 1982). The shift in vegetation from *Sphagnum*-dominated communities to vascular plant-dominated ones follows chemical conditions (pH and nutrients) along the gradient, from an ombrotrophic bog to a minerotrophic fen (Rydin and Jeglum, 2006). The metabolic pathways for methanogenesis differ between types of peatland (see Section 3.2), and microbial diversity increases along the gradient from ombrotrophic to minerotrophic peatland types (Galand et al., 2005; Juottonen et al., 2005).

The spatial hydrological conditions and plant types of peatlands define their microtopography (Figure 1), with a gradient from hollows (depressions always covered by water) to lawns (often inundated) and hummocks (dry elevations up to 50 cm) (Rydin, 1986; Fisk et al., 2003). Lawns have higher methane emission rates and less vascular plant cover than hummocks (e.g. Svensson and Rosswall, 1984; Saarnio et al., 1997; Lai, 2009). The microtopography formation results in different microhabitats, characterised by different *Sphagnum* mosses and vascular plants (Saarnio et al., 1997; Rydin and Jeglum, 2006; Robroek et al., 2009). The composition of specific microbial guilds (e.g. methanogens and methanotrophs), along with bacterial community composition as a whole, also differ between microhabitats (Galand et al., 2003; Kotiaho et al., 2012; Robroek et al., 2014; Juottonen et al., 2015). Nonetheless, the effect of microtopography on microbial communities is site-dependent, and climatic conditions seem to be an important factor that affects community structure (Yavitt et al., 2012; Robroek et al., 2014; Juottonen et al., 2015).

Figure 1. A photograph showing hummock-lawn microhabitats. The hummock microhabitat is located where Dr. Bjorn J.M. Robroek is sitting, while the lawn microhabitat is where he is measuring the chlorophyll content of the grass. Photograph by Magalí Martí Generó, printed with Dr. Robroek's consent.

3.2. Microbial degradation of organic matter in peatlands

In peatlands, there is a vertical stratification of the microbial energy-metabolism that is governed by the water level (Rydin and Jeglum, 2006; Artz, 2009). As the availability of O₂ drastically declines with depth, the degradation of organic matter is constrained to metabolic pathways that utilise alternative terminal electron acceptors and fermentative processes coupled to methanogenesis (Figure 2). The upper horizon (acrotelm), located above the water level, is mainly oxic, and organic matter there is degraded largely by aerobic microorganisms (fungi and bacteria). This leads to CO₂ production, and up to 50% of the CH₄ that diffuses towards the atmosphere is oxidised to CO₂ by methanotrophs (Conrad, 1996). The bottom and most voluminous horizon (catotelm), located below the water level, is constantly anoxic, and is dominated by anaerobic degradation that takes place at lower decomposition rates than aerobic degradation. Under anaerobic conditions, organic matter is degraded to the end products CH₄ and CO₂ (Figure 2). The interface (mesotelm) between the acrotelm and the catotelm is periodically oxic and periodically anoxic, and is the most metabolically active horizon; here, both aerobic and anaerobic metabolisms meet. The anaerobic decomposition process of organic matter consists of complex and interconnected (syntrophic and competitive interactions) successive steps involving different microorganisms, including fermenters, acetogens, syntrophs, syntrophic acetate-oxidising bacteria (SAOB), and methanogens (Conrad, 1999; Whalen, 2005; Bridgham et al., 2013). In brief (Figure 2), the organic matter that enters the anoxic horizon consists mostly of polysaccharides (mainly cellulose and hemicellulose), proteins, and lignin. The majority of the biopolymers are hydrolysed into monomers (glucose, amino acids) by extra-cellular hydrolytic enzymes, which are produced and excreted by fermenting bacteria and fungi. These monomers are subsequently fermented into fatty acids, alcohols (e.g. acetate, ethanol, propionate), hydrogen (H₂), and CO₂. Acetate can also be directly produced as an end product of the fermentation of monomers, or from H₂ and CO₂ by acetogenic bacteria (homoacetogenesis). The fatty acids and alcohols are fermented into acetate, H₂, and CO₂ by syntrophic bacteria, and function as substrates for methanogens. Syntrophic bacteria produce H₂ but, at the same time, need a low partial pressure of H₂ for fermentation to be thermodynamically favourable. Thus, as H₂ is utilised by methanogens as an electron donor, both guilds live in syntrophy (Schink, 1997).

Several microbial taxa with the metabolic potential for organic matter degradation, as well as their key enzymatic pathways, have been identified in various types of peatland ecosystem

(Hamberger et al., 2008; Drake et al., 2009; Wust et al., 2009; Tveit et al., 2013; Lin et al., 2014; Schmidt et al., 2015; Tveit et al., 2015; Schmidt et al., 2016; Juottonen et al., 2017). Generally, the most abundant phyla detected in peat samples comprise several taxa that are able to produce energy through fermentative processes, i.e. Actinobacteria, Acidobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Spirochaetes, and Verrucomicrobia. However, microbial community composition and metabolic potential differ among peatland types.

Methanogenesis is the last step of the anaerobic degradation of organic matter that leads to CH₄ formation. In principle, it takes place when alternative terminal electron acceptors such as O₂, NO₃⁻, Fe³⁺, and SO₄²⁻ are limited or depleted, which is a common situation in peat (Conrad, 1999; Bridgham et al., 2013). Members of the phyla Actinobacteria and Proteobacteria have been related to the anaerobic respiration in peat (Tveit et al., 2013). There are three main metabolic pathways for CH₄ formation based on the substrate that is utilised, and all involve the conversion of a methyl group to CH₄ (Garcia et al., 2000; Deppenmeier, 2002; Ferry, 2002; Liu and Whitman, 2008): (1) Hydrogenotrophs reduce CO₂ (electron acceptor) with H₂ (electron donor); some are able to utilise formate (HCOOH) as a source of both CO₂ and H₂. The orders *Methanomicrobiales*, *Methanococcales*, *Methanopyrales*, and *Methanobacteriales* (except for the genus *Methanosphaera*) are obligate hydrogenotrophic methanogens. (2) Acetotrophs cleave acetate (C₂H₃O₂⁻) into methyl and carbonyl groups. The oxidation of the carbonyl group into CO₂ is coupled to the reduction of the methyl group into CH₄. The acetotrophic pathway is carried by the order *Methanosarcinales*, which is the most metabolically versatile order aside from the family *Methanosaetacea*, which is an obligate acetotroph. (3) Methylotrophs utilise methylated compounds such as methanol, methylamines, and methylsulfides, which act as both electron donor and acceptor. However, H₂ is also used as an electron donor. Methanogens that belong to the order *Methanosarcinales* and the genus *Methanosphaera* (order *Methanobacteriales*) are methylotrophs.

The hydrogenotrophic pathway – which is the most energetically favourable reaction (-135.6 KJ/mol CH₄ at standard conditions) – is more common in acidic ombrotrophic bogs, while the acetotrophic pathway – the least energetically favourable reaction (-31 KJ/mol CH₄ standard conditions) – is more common in minerotrophic habitats (Horn et al., 2003; Kotsyurbenko et al., 2004; Galand et al., 2005; Kotsyurbenko et al., 2007).

Figure 2. A schematic overview of the C and CH_4 cycling of peatland ecosystems. Solid arrows show the primary successive steps of the anaerobic decomposition process, which are mediated by different groups of microorganisms (blue boxes). Red circles denote the intermediate C pools. Red and green dashed lines represent the net flux of CH_4 and CO_2 gases, respectively. ΔG represents the metabolic energy constraints, illustrated by the decrease in the amount of free energy available with increasing depth. Adapted from Conrad (1999) and Bridgham et al. (2013).

Some archaea, which are phylogenetically related to methanogens, perform anaerobic oxidation of methane (AOM) via reverse methanogenesis coupled to alternative electron acceptors (i.e. sulfate-dependent, nitrate/nitrite-dependent, and iron-/manganese- or humic acid-reduction), reviewed in Cui et al. (2015) and Timmers et al. (2017). AOM has been reported in peatland ecosystems (Smemo and Yavitt, 2007; Shi et al., 2017).

Methanogenesis in peatlands is primarily regulated by the availability and degradability of organic matter, which is determined by the primary production and composition of the plant community (Svensson and Sundh, 1992; Bridgham et al., 2013). Methanogens are dependent on temperature, with optimal methanogenic activity occurring between 20 and 35°C, meaning that temperature is a limiting factor in northern peatlands (Svensson, 1984; Bergman et al., 1998). It has been shown that temperature strongly influences the composition and methane production rates of the methanogenic community in peatlands (Liu et al., 2012; Blake et al., 2015), and that the impact of temperature on methanogens is modulated by moisture regimes and S deposition (Vile et al., 2003; Gauci et al., 2004; Turetsky et al., 2008; Peltionemi et al., 2016). In the presence of sufficiently high concentrations of sulfate (electron acceptor of sulphate-reducing bacteria [SRB]), methanogens may be outcompeted by SRB due to the fact that both guilds utilise H_2 as electron donor, and SRB have a higher affinity for H_2 (Abram and Nedwell, 1978; Kristjansson et al., 1982).

3.3. Interactions between plant and microbial communities in peatlands

While microbial communities have long been seen as key drivers of biogeochemical processes, the interconnection between plant and soil microbial communities is being increasingly recognised as a major component of ecosystem processes such as C dynamics (Wardle et al., 2004; Singh et al., 2010). Plants regulate soil microbial growth by providing organic matter into the soil (C input into the system) through root exudates and plant litter. The decomposition and mineralisation of this organic matter, carried out by the soil microorganisms (C output of the system), in turn regulates plant growth by determining the availability of soil nutrients. As regards peatlands, consensus is building that the structure and activity of microbial communities, with direct consequences for C and nutrient-transformation dynamics, as well as CO_2 and CH_4 fluxes, are related to the plant community composition (Fisk et al., 2003; Rooney-Varga et al., 2007; Bragazza et al., 2012; Jassey et al., 2013; Jassey

et al., 2014; Kupier et al., 2014; Robroek et al., 2015; Yavitt and Williams, 2015). Sedges such as *Carex spp.* and *Eriophorum spp.*, for example, are well adapted to anoxic conditions as a result of the fact that they are able to transport oxygen into their roots through aerenchymal tissues (Armstrong et al., 1991). The presence of root systems within the anoxic zone has a direct effect on microbial activity, in that these systems transport O₂ and supply easily degradable root exudates. These may stimulate the decomposition of the recalcitrant fractions of the organic matter that is yet to be degraded, ultimately promoting methanogenic activity, meaning that sedge-dominated peatlands produce more CH₄ than *Sphagnum*-dominated ones (Joabsson et al., 1999; Lai, 2009; Nilsson and Öquist, 2009; Preston et al., 2012). Climatic change scenarios and increased rates of atmospheric nutrient deposition are projected to have direct effects on plant communities and, consequently, microbial communities in peatlands (Limpens et al., 2011; Bragazza et al., 2013). Ultimately, such changes may result in feedbacks on the climate system. It is thus of great importance to investigate the effects of climate and environmental change on the interactions between plant and microbial communities in peatlands.

3.4. Molecular detection and characterisation of microbial communities

The primary challenge in elucidating the link between microbial community composition/function and ecosystem processes lies in the methodology (Prosser et al., 2007; Prosser, 2015; Graham et al., 2016; Widder et al., 2016). Until the mid-1980s, microbes were usually studied using culture-dependent methods, but laboratory cultivations have been shown to be insufficient as regards characterising the vast microbial richness of the biosphere, with estimations suggesting that < 1% of all microorganisms are cultivable (Staley and Konopka, 1985). In addition, it has recently been shown that the common practice for agar medium preparation generates byproducts that are harmful to many microorganisms (Tanaka et al., 2014).

The introduction of nucleic-acid based methods (molecular methods) based on ribosomal RNA (rRNA) genes, which provide evolutionary and taxonomic information, together with the invention of Sanger sequencing in 1977, allowed the diversity of microorganisms in the environment to be studied more accurately (Handelsman, 2004). The 16S rRNA gene encodes the small subunit of ribosomal RNA, which is essential for protein synthesis and well conserved and ubiquitous across prokaryotes. Thus, it is well suited for and extensively used in the characterisation of microbial phylogeny and taxonomy, while 16S rRNA is used to

estimate microbial activity, based on the assumption that activity and growth are the same. However, it has been shown that 16S rRNA is often not ideal for characterising microbial activity in a community, mainly because concentration of 16S rRNA and growth rate differ among taxa, thereby they are not necessarily correlated with activity (Blazewicz et al., 2013). This means that the relative abundance of 16S rRNA in a community is not necessarily indicative of which taxa are relatively more active. In addition, 16S rRNA is also present in dormant cells. Moreover, several microbial activities, such as cell maintenance or cell motility, are not necessarily related to growth (Blazewicz et al., 2013). The use of marker genes that encode for specific functions is often preferred when assessing microbial activity, as some of the microbial genes that encode for enzymes are unique to specific processes. For example, the methyl-coenzyme M reductase (MCR, EC 2.8.4.1) catalyses the final reaction in methanogenesis, wherein the methyl group is reduced and CH₄ is consequently formed. MCR is a key enzyme in methane formation, and is unique to and ubiquitous among methanogens (Deppenmeier, 2002). The gene encoding for the α -subunit of the MCR enzyme (*mcrA*) has been broadly used as a marker gene to study the abundance of methanogens, while the *mcrA* transcript (gene expression) has been used to study active methanogens in the environment (reviewed in Andersen et al., 2013).

In the last decade, the development of next-generation sequencing (NGS) technologies that enable massive parallel sequencing (high-throughput sequencing) has revolutionised the microbial ecology field (Margulies et al., 2005), making it possible to characterise the entire composition of a microbial community (phylogeny and taxonomy) and genetic pool of a sample (Thomas et al., 2012; Vincent et al., 2016). The use of these techniques has provided a new understanding of the functional and ecological roles of individual microbial taxa. Metagenomics enable the direct characterisation of the genetic pool (genomes) of an environmental sample through sequencing of all of the DNA fragments that it contains. To obtain insight into microbial activity, the gene expression can be characterised by sequencing the messenger RNA (mRNA) in a process that is referred to as 'metatranscriptomics' (Carvalhais et al., 2012; Thomas et al., 2012; Prosser, 2015). However, NGS technologies are not exempt of limitations: They are affected by the common bias problems of molecular techniques, such as nucleic acid extraction, polymerase chain reaction (PCR), and sequencing errors (Knief, 2014). The classification of sequences into known operational taxonomic units (OTUs) and functions depends on available database coverage (Thomas et al., 2012). It should also be noted that high-throughput sequencing data is presented in the form of relative abundance, and it is thus important to be careful when relating differences in the relative abundance of

functional genes to potential activity rates and processes in ecosystems (Prosser, 2015). The comparison of functional genes by their relative abundance means that an increase in one gene reduces the relative abundance of others, but not necessarily their absolute abundance. For example, increased S deposition in peatlands might stimulate S reduction, and therefore increase the abundance of functional genes related to that process. Increases in such genes imply a reduction in the relative abundance of other genes, such as those involved in methanogenesis. Additionally, comparing and integrating data from different studies is not straightforward due to the general lack of standardised methods for undertaking this process (Philippot et al., 2012).

4. Methods

4.1. Site selection and sample collection

4.1.1. Ombrotrophic raised bogs (Papers I, II)

Ombrotrophic raised bogs constitute optimal sites for studying the effects of atmospheric nutrient deposition due to their being nutrient-poor, with rainfall being the only supply of water and nutrients. The microtopography of these bogs, as shaped by the hummock-lawn contrast along the water table gradient and the consequent formation of microhabitats, makes those ecosystems ideal for investigating the influence of site-intrinsic conditions (local-scale) on above- and below-ground biotic communities.

To assess microhabitat influences and compare these to geo-climatic influences on microbial communities (Research Objective 1), peat samples were collected from European ombrotrophic raised bogs, as is described in Papers I and II. These two studies were a part of a European collaboration project within ERA-net: 'Pollution, Precipitation and Temperature Impacts on Peatland Biodiversity and Biogeochemistry'. Within this ERA-net project, 59 ombrotrophic raised bogs were selected along an atmospheric N deposition, climate, and latitudinal gradient. Average data from 2005 to 2009 of atmospheric deposition (total N and S) and bioclimatic factors (mean annual temperature, mean annual precipitation, temperature seasonality, and precipitation seasonality), which were used to describe environmental differences between the sites (Table 2 in Section 5.1), were obtained from the European Monitoring and Evaluation Programme (EMEP) and the WorldClim database, respectively (Hijmans et al., 2005). In order to assess the influence of geo-climatic conditions, three sites (Degerö Stormyr, Cena Mire, and Dosenmoor) that differed in terms of climatological conditions and atmospheric N and S depositions were selected for the study presented in Paper I (Figure 3 and Table 2). Two other sites (Store Mosse and Lille Vildmose) and Degerö Stormyr were selected for the study presented in Paper II.

In Paper I, in order to investigate how the link between plants and microorganisms is affected by differing environmental conditions (Research Objective 3) and microtopography (Research Objective 1), plant species abundance was recorded at each hummock-lawn microhabitat ($n = 6$ per site) in a 0.5 m² area. Peat extraction was then performed for the purpose of microbial analysis at the same quadrants, with peat samples being collected from above the water table level (AWTL) and below the

water table level (BWTL). A similar approach was used in order to elucidate how methanogens are affected by site-intrinsic microhabitats (Research Objective 1; Paper II), with peat samples being collected following the microtopographic gradient of the hummock-lawn mosaic north to south (Figure 1 in Paper II). Thus, five microtopographic gradients were sampled per site, with peat samples being collected from both AWTL and BWTL.

Figure 3. The location of the peatland sites studied in Papers I (Degerö Stormyr, Cena Mire, and Dosenmoor) and II (Degerö Stormyr, Store Mosse, and Lille Vildmose).

4.1.2. Field experiment: Degerö Stormyr (Papers III, IV)

In order to avoid problems relating to variation between samples arising from the differing geo-climatological conditions of sites and other site-related conditions - such as plant distribution, water regimes, peat age, and pH - peat samples were collected from an experimental site in a *Sphagnum*-dominated oligotrophic mire at Degerö Stormyr, northern Sweden (64°11'N, 19°33'E, 270 m above sea level). The experimental site simulates warming and increased N and S depositions, and by avoiding the above-mentioned variables, allows investigations of the specific effects of these three perturbations on the mire (Research Objective 2). The experimental site (Figure 4), which was established in 1995, consists of a full factorial experimental design of 20 plots (all of 2 x 2 m, and surrounded by a 0.5 m deep polyvinyl chloride

frame 0.5 m deep). The full factorial design includes two levels of N (ambient [2 kg] and addition of $[\text{NH}_4\text{NO}_3]$ to reach 30 kg N ha⁻¹ yr⁻¹), two levels of S (ambient [3 kg] and addition of $[\text{Na}_2\text{SO}_4]$ to reach 20 kg S ha⁻¹ yr⁻¹), and two levels of warming (ambient or with greenhouse covers, performed by covering the plots with perforated plastic film, supported by transparent polycarbonate frames, during each summer). Each experimental combination was duplicated. The levels of addition of N and S represent the deposition levels of these elements in south-western Sweden at the start of the experiment (Granberg et al., 2001). For a detailed description of the experiment see Granberg et al. (2001). For details regarding the treatment effects on vegetation composition and CH₄ fluxes, see Wiedermann et al. (2007) and Eriksson et al. (2010a,b). In brief, CH₄ emissions and production rates have increased with (simulated) increased N deposition, and decreased with simulated warming after a decade of experimental manipulations. The vegetation community has shifted towards vascular plant dominance with both increased N deposition and warming.

Figure 4. A photograph of the Degerö Stormyr site, taken in August 2013 (by Magalí Martí).

This field experiment was thus judged to be ideal for investigating the long-term (18 years) effects of warming and increased N and S depositions on peat microbial communities (Research Objective 2). Samples were collected at five different depths

in order to cover the peat redox profile that constrains the aerobic metabolism in the upper peat horizon (acrotelm), the anaerobic metabolism in the deeper peat horizon (catotelm), and an intermediate zone (mesotelm) where both types of metabolic process occur (Papers III, IV).

4.2. Molecular methods

Peat samples present a particular challenge with regard to the use of molecular techniques due to the overall low microbial biomass and peat matrix conditions. The combination of high acidity, high content of humic acid, high water content (up to 90%), high capacity to form complex with nucleic acids, high content of phenols, and other co-extractable organic matter, affects the quality of the nucleic acid extraction and the enzyme reactions that form the basis of the molecular techniques (Vaughan and Ord, 1982; Crecchio and Stotzky, 1998). During the work that is presented in this thesis, various methods were used to investigate the structure and function of microbial communities (Figure 5). Sample preparation was the same for all of the molecular methods used, and involved homogenisation followed by total nucleic acid extraction. All of the methods are based on the PCR technique, which amplifies a specific sequence of DNA or complementary DNA (cDNA) across several orders of magnitude in order to enable detection.

Quantitative PCR (qPCR)

This technique is derived from conventional PCR, and enables the real-time detection of a fluorescent signal of the amplified sequence throughout each amplification cycle (Smith and Osborn, 2009). This allows the initial amount of DNA or cDNA in samples to be determined. Thus, qPCR is a widely-used method for quantifying the amount of genes that is present in a sample, and was used in the work described in Papers II and IV to quantify the amount of the *mcrA* gene and *mcrA* transcript per gram of soil.

Terminal restriction length polymorphism (T-RFLP)

This technique is a fingerprinting method that is based on the position of the terminal restriction fragments (short sequences recognised by restriction enzymes) of a specific gene that has been amplified using PCR (Dunbar et al., 2001). T-RFLP is a good and cost-effective method of determining the diversity of a specific gene present in a sample, and was used in the work described in Paper II to characterise the diversity of the *mcrA* gene and *mcrA* transcript in peat samples.

Figure 5. Overview of the molecular methods used in the work presented in this thesis.

Amplicon sequencing

This technique enables the entire microbial composition of a sample to be analysed by sequencing the 16S rRNA gene across multiple species (Vincent et al., 2016). It is frequently used in studies of microbial ecology due to the fact that it reveals the microbial phylogeny and taxonomy present in a sample. In the work described in this thesis, both 16S rRNA gene amplicon sequencing (Paper III) and 16S rRNA amplicon sequencing (Papers I, III) were used to characterise the prokaryotic taxonomic composition of the peat samples.

Shotgun sequencing

This technique enables genomes contained in an environmental sample to be analysed by sequencing the entire genetic pool of the microbial community (Thomas et al., 2012). It was used to characterise the entire genetic pool (i.e. functional potential) of each peat sample taken from the experiment at Degerö Stormyr (Paper III).

4.3. Statistical analysis

Analysis of variance

Permutational multivariate analysis of variance (Adonis) is a nonparametric equivalent to the analysis of variance (ANOVA), and is used to test for significant differences between two or more groups based on a dissimilarity matrix (Anderson, 2001). Adonis was used to test the hypothesis that microbial communities from the same sites were more similar in terms of composition to one another than to communities from different sites (Research Objective 1; Papers I, II). It was also used to test the hypothesis that microbial community taxonomic and functional composition had been affected by warming and increased N and S depositions (Research Objective 2; Paper III). Microbial community richness and evenness (alpha-diversity) were tested for differences between sites (Paper I) and treatment effects (Paper III) using ANOVA.

Correlations and relationships

Pearson correlation was used to analyse the degree of association between abundance of methanogens and edaphic variables (Paper II), abundance of plants (Paper IV), and CH₄ production rates (Paper IV). Multiple linear regression (MLR) was used to test the hypothesis that individual metabolic traits are affected by warming and increased N and S depositions, and that the effects of these perturbations interact with one another (Research Objective 2; Papers III, IV). Distance-based redundancy analysis (db-RDA) (Legendre and Anderson, 1999) is a constrained linear ordination method that combines principal component analysis (PCA) and MLR. It constrains the main components (response variables) such that they are linear combinations of the explanatory variables, where MLRs are fitted to find the best solution. This methodology was used to explore possible multiple linear correlations between the response variable (microbial communities) and the explanatory variables (edaphic variables, environmental variables, and vegetation community composition; Papers II, III). Mantel test calculates correlation coefficients between two (dis)similarity or distance matrices (Mantel, 1967), and was used to test the relationship between microbial community composition and climatic and nutrient factors (Paper I). Correspondence between the data sets of Paper III was investigated using Procrustes superimposition combined with a randomisation test (Peres-Neto and Jackson, 2001).

Ordination methods

Non-metric multidimensional scaling (NMDS) maps the pairwise dissimilarity of ranked distances to a k-dimensional ordination space, where the distance between

objects corresponds to their (dis)similarity (Minchin and Peter, 1987). It is an iterative numerical procedure for finding the best ordered solution. NMDS was used to visualise the distribution of communities across sites (Paper I) and treatments (Paper III), as well as to assess the co-variation between community composition and CH₄ production/oxidation rates by fitting the process data onto the ordination (Paper III).

4.4 Co-occurrence network

Co-occurrence networks were applied to detect co-presence or exclusion of prokaryotes and plants, indicative of the ecological interactions structuring the communities. The networks were obtained using a correlation matrix, where each species (or OTUs) was pair-wise related to the others (Pearson's correlation) (Berry and Widder, 2014). Significant ($p < 0.05$) r values ≤ 0.6 were considered to demonstrate as negative relationships (-1), and $r \geq 0.6$ as positive relationships (+1), (Williams et al., 2014). Each Pearson's correlation matrix then was transformed into a binary matrix based on the presence (+1 or -1) or absence (0) of links. Finally a set of network features was evaluated:

Network topography

Network indices were used to evaluate the topological properties of the co-occurrence networks: *Node degree* (D) is the number of links of a node. *Betweenness centrality* (BC) evaluates the importance of a node in connecting the different clusters within a network (Freeman, 1977). BC is considered to be indicative of network resilience (Newman, 2003). *Closeness* (C) calculates the average distance of a given node to any other node. *Shortest path length* (SP) is the shortest path between any two nodes with the least links between them.

Network dissimilarity

Differences in species interactions between networks (β_{WN}), derived from differences in species composition and shared species interactions between pairs of network (Poisot et al., 2012), was used to quantify the degree of (dis)similarity between networks.

Keystone species

Species that have a disproportionate deleterious effect on the network properties upon their removal are considered keystone species. These were defined by calculating the generalized keystone index (GKI), which is the mean of all scaled and standardized network indices (D, BC, C and SP).

5. Results and discussion

Microbial community dynamics are strongly affected by peat redox conditions, which in turn are determined by the water table level (see Section 3.2). Thus, the impact of environmental perturbations on microbial communities is often depth-dependent. In the discussion that follows, and where depth had an effect on the result, the depth horizons are reported as AWTL, FWTL, and BWTL, where ‘A’ denotes ‘above’, ‘F’ ‘flanking’, and ‘B’ ‘below’, corresponding to the acrotelm, mesotelm, and catotelm, respectively. If no mention is made of depth, it had no influence on the final result.

5.1. Local- and large-scale variability of microbial communities (Papers I, II)

This section assesses the role of the microhabitat within sites (local-scale) in relation to site locations (large-scale) with regard to effects on archaeal and bacterial community composition (Paper I) and methanogen community dynamics (Paper II). Peat samples (for microbial analysis) and pore water (for nutrient content analysis) were collected from five ombrotrophic bogs of differing atmospheric deposition and climatic conditions (Table 2).

Table 2. The climatological and atmospheric deposition variables used to describe the differences between the study sites. MAT: Mean annual temperature. MAP: Mean annual precipitation. TS: Seasonality in temperature. PS: Seasonality in precipitation.

		Degerö Stormyr	Store Mosse	Lille Vildmose	Cena Mire	Dosenmoor
Location		Northern Sweden	Southern Sweden	Denmark	Latvia	Northern Germany
Paper		I, II	II	II	I	I
Climate	MAT (°C)	1.2	6.2	7.6	6.4	8.0
	MAP (mm)	730	970	680	710	760
	TS (°C)	9.3	6.1	6.6	7.9	5.9
	PS (%)	26	22	22	31	19
Deposition (mg m ² yr ⁻¹)	Total N	130	620	680	760	1260
	Total S	83	290	400	480	480

*TS: amount of temperature variation over a given year based on the standard deviation of monthly temperature averages.

**PS: ratio of the standard deviation of the monthly total precipitation to the mean monthly total precipitation (coefficient of variation).

5.1.1. Spatial variability of Archaea and Bacteria community compositions

The prokaryote community composition differed significantly across sites (large-scale), while the effect of the hummock-lawn microhabitat (local-scale) on the community turnover was site-dependent (Table 2 and Figure 3 in Paper I). At the AWTL horizon, site explained 23% and 25% of the archaeal and bacterial community variance, respectively. The turnover of the archaea community was only related to the difference in nutrient content ($r = 0.2$, $p < 0.05$; NO_3^- , NH_4^+ , PO_4^{3-} , SO_4^{2-}) among sites, while that of the bacterial community was related to nutrient content ($r = 0.6$, $p < 0.05$) and to climatic factors ($r = 0.2$, $p < 0.05$; MAT, MAP, TS, and PS). The bacterial community also had a high degree of correspondence with the plant community ($r = 0.8$, $p < 0.001$). At the BWTL horizon, the site had a stronger effect on the prokaryote community composition compared to the AWTL horizon, explaining 40% and 37% of the archaeal and bacterial variance, respectively. The bacterial community turnover was only related to climatic factors ($r = 0.3$, $p < 0.001$), while that of the archaeal community was related equally to both nutrient content and climatic factors ($r = 0.3$, $p < 0.001$). Both bacterial and archaeal communities displayed a high concordance with the plant community at BWTL (bacteria $r = 0.9$ and archaea $r = 0.8$, $p < 0.001$). The plant community turnover was also strongly influenced by site (which accounted for 40% of the variance), and correlated with the climatic factors ($r = 0.6$, $p < 0.05$) and, to a lesser degree, the nutrient content ($r = 0.3$, $p < 0.05$). Due to the nutrient-poor conditions in these peatlands, additional nutrient deposition is likely to be immediately taken up by plants, which is the likely explanation for why the BWTL communities, compared to the AWTL, were less related to nutrient content. The impact of climatic factors on the prokaryote community was more pronounced at the BWTL horizon. The plant community also showed stronger correlations with the climatic factors compared to the nutrient content in the peat, indeed clear effects of climatic perturbations on plant communities have been observed in peatlands (Wiedermann et al., 2007; Limpens et al., 2011; Bragazza et al., 2013). It is thus, suggested that the effect of the climatic factors on the BWTL communities may be mainly indirect through induced changes to plant community.

Although the observed microbial community turnover across sites was clearly related to climatic factors as well as plant composition and the nutrient content of the peat, dispersal limitation may also potentially constrain the microbial composition (Ramette and Tiedje, 2007; Landesman et al., 2014). However, according to findings by Fierer and Jackson (2006) and Landesman et al. (2014), we expect that dispersal limitation has a minor effect on the peat microbial community composition. In their efforts to determine the best predictors of microbial community

composition and structure in relation to describing the large-scale spatial variation of microorganisms (i.e. microbial biogeography) they consistently found that environmental factors – primarily pH – seem to be the main drivers of community composition among different soil ecosystems, and that dispersal limitation (i.e. geographical distance) contributes to a minor extent (Fierer and Jackson, 2006; Landesman et al., 2014). In addition, soils from different sites with similar environmental characteristics that determine species habitat (ecological niche) have similar bacterial communities, regardless of geographical distance (Fierer and Jackson, 2006). Moreover, particularly in peatlands, the methanogenic community composition turnover across six peatlands in North America was found to be strongly related to pH and temperature, and weakly related to geographical distance (Yavitt et al., 2012). Taken together, these results point to the clear impact of climatic factors on microbial community composition.

5.1.2. Spatial variability of methanogenic archaeal community

The abundance of *mcrA* gene (bulk methanogens) and *mcrA* transcript (active methanogens) was significantly influenced by the site factor (explaining 19% and 9% of the variance, respectively; Table 3 in Paper II). The highest abundance of active methanogens was observed at the site with the highest temperature and that received the greatest amount of N deposition, while the lowest abundance of active methanogens was observed at the site with the lowest temperature and least N deposition (Figure 2 in Paper II). The mean daily amplitude soil temperature (for June to September 2009) correlated with the abundance of the *mcrA* gene ($r = 0.47$, $p < 0.01$). There was a correlation between the abundance of *mcrA* transcript and the amount of NO_3^- and dissolved organic carbon (DOC) in the pore water ($r = 0.3$, $p < 0.01$). When the influence of the site was not factored in, an effect of the microhabitat on both the *mcrA* gene and its transcript was observed for Degerö Stormyr (explaining 7% and 6% of the variance, respectively) and Lille Vildmose (explaining 9% and 25% of the variance, respectively). Overall, there was a greater abundance of the *mcrA* gene and its transcripts in the lawn microhabitat than the hummock one. This was expected due to the location of the water table to the peat surface, which is known to result in higher methane production and emissions from lawn microhabitats (e.g. Svensson and Rosswall, 1984; Granberg et al., 1999). These findings were in line with those of a parallel study conducted at the same sites and sampling locations, in which the site was the main factor in the variation in plant and bacterial community composition, and the influence of the microhabitat on plant

and microbial communities was only revealed when the site effect was partialled out (Robroek et al., 2014).

The methanogenic community composition was only influenced by the site with no effect of the microtopography. Similar results were found by Juottonen et al. (2015), which investigated the effect of microtopography on the methanogenic diversity of four boreal bogs. They concluded that the effects of microtopography are site-dependent, which supports the results in Papers I and II. On the contrary, the findings from Galand et al. (2003) showed that the methanogenic community of a minerogenic oligotrophic fen was affected by its microtopography. This discrepancy indicates that the spatial distribution of methanogens along the microtopography may be influenced by the ombrotrophic-minerotrophic gradient. In the present study, median soil temperature of the growing season from June to September and DOC content across the sites explained the variation in methanogenic community composition by 26% and 12%, respectively. In accordance with these results, a previous study showed that mean summer air temperature and DOC accounted for almost half of the variance in the methanogenic community composition across five different wetlands, including one peatland, in China (Liu et al., 2012). The concentration of DOC in peat is suggested to be related to the abundance of vascular plants coupled to a decline in *Sphagnum* cover and increase in phenolic compounds (Fenner et al., 2007; Bragazza et al., 2013).

In line with the general notion that the hydrogenotrophic pathway is the most common in acidic ombrotrophic bogs (see Section 3.2), the family *Methanoregulaceae* was found to dominate in the three sites reported in Paper II. Furthermore, the composition of the methanogenic community derived from the 16S rRNA was also dominated by the hydrogenotrophic pathway in the three sites reported in Paper I, as the family *Methanobacteriaceae* accounted for 84% of all classified sequences across the three studied sites. However, in the in-situ experiment (Paper III), the treatment plots receiving N addition or warming revealed a clear dominance by the family *Methanosarcinaceae*, accounting for 100% of all classified 16S rRNA sequences. The order *Methanosarcinales* is the most metabolically versatile (see Section 3.2). Still the hydrogenotrophic family *Methanobacteriaceae* dominated the methanogen community in the treatment plots receiving S addition, representing 100% of all the classified 16S rRNA sequences. The main difference of the in-situ experiment is that long-term warming and N addition have promoted vascular plants (primarily *Eriophorum vaginatum* L., *Andromeda polifolia* L. and *Vaccinium oxycoccos* L.), which will indirectly and directly affect the methanogens. Thereby, a plausible explanation is that the plants via their root exudates provide acetate, which would directly

promote acetotrophic methane production. This is supported by Ström et al. (2003), who showed that acetate is a main labile part of the root exudate from the sedge *Eriophorum scheuchzeri* (Hoppe) and that the rate of acetate formation is dependent on the photosynthetic rates. The lack of *Methanosarcinales* in the S-amended plots is likely due to the low abundance of vascular plants in these plots. Thus, it seems as if the presence of *Methanosarcinales* is related to the occurrence of vascular plants in mire systems. This is corroborated by the results from studies of nutrient gradients in mire ecosystems, where acetotrophic methanogens often dominate (cf. Galand et al., 2005; Juottonen et al., 2005; Galand et al., 2010). The long-term of N addition and warming treatments seem to have moved the oligotrophic poor fen type system at Degerö Stormyr to a more nutrient-rich habitat, which seems to be confirmed by the development of the methanogenic community structure.

5.2. Effects of warming and increased atmospheric N and S depositions on microbial communities (Papers III, IV)

The results presented in this section were obtained from two studies based on a long-term in-situ experiment that simulates warming and increased atmospheric N and S depositions (Papers III, IV). Here the responses of the prokaryote taxonomic and functional composition (Paper III) and active methanogens (Paper IV) to these perturbations are assessed.

5.2.1. Microbial community responses to warming and increased N and S depositions (Paper III)

The taxonomic composition of the microbial community significantly shifted in response to the three perturbations after a long-term, 18 years, of field simulations (Table 1 and Figure 1 in Paper III). Among the three perturbations, increased N deposition had the highest impact on the microbial community composition, significantly affecting it throughout all of the depth horizons and contributing the most to the explained variance (from 8% to 25 %, Table 1 in Paper III). The turnover in community composition was clearly different for each perturbation; this is shown by an NMDS plot (Figure 6), where the proximity of sites indicates similarity in terms of microbial composition. The taxonomic composition derived from the 16S rRNA gene was strongly correlated with the functional potential (assessed by sequencing the entire genetic pool; $r = 0.87$, $p < 0.01$). However, the functional potential of the microbial community, was only statistically significantly affected by

simulated N deposition, which explained 14% of the variance (Table 1 in Paper III). The general lack of significant responses to the perturbations is likely explained by the composition of the genetic pool, in which core (housekeeping) genes, rather than specific functional ones, account for most of the metagenome. These core genes encode for the central metabolism and anabolism that are common to most bacteria (e.g. nutrient uptake, energy retrieval, and synthesis of microbial biomass), while the functional genes encode for energy metabolism (Medini et al., 2005; Chubukov et al., 2014).

The lack of response of the functional potential to the perturbations, in complete contrast to the clear changes in taxonomic composition, indicates a high degree of functional redundancy among the microbial community members selected for under the various perturbation conditions. Functional redundancy has also recently been suggested among the peat fermentative taxa of an oligotrophic, a mesotrophic and an eutrophic peatland, as different microbial communities in these peatlands were found to perform similar anaerobic energy-metabolism processes (Hunger et al., 2015). Plant community composition has also been found to have a high degree of functional redundancy in peatlands (Robroek et al., In review). There were significant correlations between microbial taxonomic composition, especially as derived from 16S rRNA, and CH₄ production and oxidation rates (Table 2 in Paper III). The interval of six years between the process rate measurements of Eriksson et al. (2010a) and the microbial community investigations (Paper III) imply that the stabilization of the plots to the effects of the perturbations was sustained also to the time for the present sampling. This is strongly corroborated by the development of the plant distribution patterns, which are largely unchanged since then. This suggests that the newly established community composition that was selected under each set of perturbation conditions is related to the anaerobic degradation processes that lead to methanogenesis. In support to this, peat microbial dynamics have found to respond rapidly to a simulated temperature increase (from 1 to 30°C) with consequent higher rates of CH₄ production with higher temperatures, in a laboratory incubation study (Tveit et al., 2015).

There was a correspondence between microbial taxonomic and functional composition, and the plant community composition previously reported by Wiedermann et al. (2007) ($r=0.4$ to 0.7 , $p<0.001$; Table S1 in Paper III). The latter was also affected by long-term increased N deposition and warming, which have resulted in a shift towards vascular plants (*E. vaginatum*, *A. polifolia*, and *V. oxycoccus*) to the detriment of *Sphagnum* spp. cover. This indicates that the plant community strongly influences the composition of a microbial community and its genetic pool,

and is in line with other studies of soil ecosystems, in which shifts in the microbial community structure were due to soil-plant-microbe interactions driven by N loading (Ramirez et al., 2010; Fierer et al., 2012; Leff et al., 2015; Zhou et al., 2015; Boot et al., 2016; Zeng et al., 2016).

Figure 6. A NMDS plot showing the various treatments based on microbial community composition, derived from the 16S rRNA gene (A) and the 16S rRNA (B). Stress values: 0.09 (A); 0.12 (B). T: Temperature. S: Sulfur. N: Nitrogen. C: Control.

Relevant functional genes related to the experiment, i.e. genes coding for key steps in the anaerobic degradation of organic matter process, as well as aerobic methane and ammonia oxidation, were specifically assessed (Table S4 in Paper III). At the AWTL horizon, few genes that encode for key steps in processes such as hydrolysis (0.2%), syntrophy (2%), methanogenesis (9%), nitrate reduction (2%), sulfate reduction (7%), sulfur oxidation (3%), and N fixation (4%) showed a significant response to any of the perturbations including their interactive effects (Figure 3A in Paper III). The values listed refer to the percentage of genes that had a significant response to any of the perturbations within the pool of genes that were identified as coding for the same metabolism in the data set. The majority of the responses were connected to interactive effects, although some effects of the main perturbations were observed. In brief: plant polymer hydrolytic degradation potential decreased with warming, while sulfur oxidation potential increased. Dissimilatory sulfate reduction potential increased with simulated S deposition, while methanogenic potential decreased in response to all three main perturbations. Similar results were obtained for simulated N deposition when potential methanogenesis was investigated using qPCR analysis of the *mcrA* gene, but opposite results were found

for warming and S deposition (Figure 1A in Paper IV; see Section 5.2.2). At the BWTL horizon, few of the genes that encode for hydrolysis (0.2%), syntrophy (2%), methanogenesis (3%), nitrate reduction (2%), sulfate reduction (3%), sulfur oxidation (3%), and Fe oxidation (11%) had significant responses, mainly connected to warming and simulated S deposition (Figure 3B in Paper III). Sulfate reduction potential increased with simulated S deposition, while the rest of the genes encoding for the other metabolic pathways decreased in response to warming and increased S deposition.

Twelve years of simulated increased S deposition has resulted in a decrease in methane production rates (Eriksson et al., 2010a) and 6 years later, concomitantly, we observed a decrease in methanogenic potential. At AWTL, the increase in SRB, as indicated by the higher relative abundance of genes encoding for sulfate reduction, likely to some extent outcompeted the methanogens. At BWTL, the observed decrease in hydrolytic and syntrophic potentials together with an increase of the sulfate-reduction potential with simulated warming and increased S deposition, imply that less organic matter is being fermented, resulting in lower amount of metabolic intermediates and consequently less substrate availability as well as electron donors (H_2) for methanogenesis. The decrease of the methanogenic potential after 18 years of warming is in line with the previous observed decrease of methane emission and production rates (Eriksson et al., 2010a). The decrease in hydrolytic and syntrophic potentials, especially BWTL, likely reflects the effect of warming on the carbon turnover, i.e. higher aerobic respiration rates at the oxic and warmer layers resulting in lower inputs of organic matter to the anoxic zone (BWTL), that might also be more recalcitrant.

In contrast to the taxonomical composition, no significant correlations were found between either process rates and overall functional potential or the specific functional genes that encode for methanogenesis and methanotrophy. This lack of correspondence may have several causes, one of which is a decoupling between gene abundance and expression (Röling, 2007; Freitag and Prosser, 2009). This decoupling is affected by the presence of dead or dormant cells from which the genes are still detected (methodological issue). The lack of correspondence may also be due to changes in gene expression that are a result of cellular activity, the regulation of which is influenced primarily by environmental conditions, enzyme concentrations, and/or substrate concentrations. Moreover, high-throughput sequencing displays data in relative abundance (e.g. genes in relation to the metagenome), and so the variation of genes in relative abundance may not represent variation in the process that they encode for. It should also be noted that the

percentage of genes, coding for the same metabolism, that their relative abundance was significantly affected by any of the perturbations was very low (< 10%). Therefore, further investigations were carried out using qPCR in order to quantify the absolute abundance of the *mcrA* gene and its transcripts (Paper IV, Section 5.2.2). The metatranscriptome was also sequenced to assess whether gene expression is indeed linked to ecosystem processes and thus constitutes a better predictor. The metatranscriptome data set will be analysed in the future.

5.2.2. Responses of methanogens to warming and increased N and S depositions (Paper IV)

The analysis of the *mcrA* gene and *mcrA* transcript using qPCR corroborated the lack of correlation between the gene and its expression. Furthermore, strong correlations between the abundance of *mcrA* transcript and CH₄ production rates were observed, in contrast to the few correlations found between the gene and CH₄ production rates. These results support above mentioned findings that suggest that there is no direct link between the relative abundance of genes and processes rates in peat, and that the prokaryote community that has established after 18 years of simulated perturbations is related to anaerobic degradation process that lead to methanogenesis (see Section 5.2.1). Thus, in the following analysis, only those results that were achieved using the gene expression (*mcrA* transcript) indicative of active methanogens are discussed. Overall, a trend towards an increase in *mcrA* transcript abundance after 18 years of simulated warming and increased N deposition was observed (Figure 1B in Paper IV), although the response was not statistically significant. In addition, no effect of increased S deposition was observed.

Long-term increased N availability has affected the plant community composition in the form of a shift from *Sphagnum* dominance to vascular plant dominance, as well as increased CH₄ production and emission (Wiedermann et al., 2007; Eriksson et al., 2010a,b). The abundance of *mcrA* transcript correlated with the rates of CH₄ production of the samples from the simulated increase in N deposition at BWTL ($r = 0.8$, $p < 0.05$; Table 1 in Paper IV). A tendency towards an increase in *mcrA* transcript abundance with an increase in N deposition in contrast to the ambient levels, was observed. A greater abundance of vascular plant results in a larger root biomass, and likely also increased root exudation (e.g. Limpens et al., 2008; Eriksson et al., 2010b; Bragazza et al., 2012). Consequently, a greater amount of easily degradable plant litter will be available, which may ultimately increase methanogenesis. However,

this increase in root abundance has been found to occur 10-15 cm below the peat surface – i.e. all of the oxic zone – as the annual average WTL is 14 cm below the surface (Granberg et al., 2001; Eriksson et al., 2010a; Olid et al., 2017). Thus, the increase in vascular plants did not result in a substantial increase in root exudation in the anoxic zone. In line with this, positive correlations between the abundance of *mcrA* transcript and vascular plants (*E. vaginatum*, *A. polifolia*, and *V. oxycoccus*) were found for AWTL and FWTL, but not for BWTL. The simulated increase in N deposition is achieved by adding NH_4NO_3 in five doses between May and September so as to reach a level of $30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. As this system is N-limited, the plants likely directly take up the NH_4NO_3 when it is added, which is supported by the fact that nitrate has only been detected in the pore water in direct conjunction with immediately following the addition of one of the five doses. Therefore, the impact of N loading on methanogens, as well as the microbial community as a whole, is likely to occur primarily in the form of induced changes in the plant community.

Simulated warming also promoted a shift from *Sphagnum* towards vascular plants; this was likely a result of changes in the water level, with a consequent increase in root biomass in the upper 10-15 cm. In contrast to increased N deposition, however, warming resulted in a decrease in CH_4 production rates and emission. The abundance of *mcrA* transcript correlated with the rates of CH_4 production under simulated warming conditions at BWTL ($r = 0.7$, $p < 0.05$; Table 1 in Paper IV). The in-situ experiment only simulates warming during the growing season, with a maximal peat temperature increase of 2-3°C at 18 cm below the surface. This means that the maximal peat temperature change occurs throughout the oxic zone, and thus likely promotes aerobic decomposition (see Section 3.2). Consequently, the organic matter left available when it reaches the anoxic zone is of lower degradability, and thus ultimately negatively affecting the methanogen community. A decrease in the hydrolytic potential BWTL that supports this conclusion was found during the work presented in Paper III, and which likely reflects lower biopolymer availability (see Section 5.2.1).

The abundance of *mcrA* transcript also correlated with the rate of CH_4 production with increased S deposition at BWTL ($r = 0.8$, $p < 0.05$; Table 1 in Paper IV). The absence of any discernible effect of increased S deposition on the *mcrA* transcript was in line with the observations of a study conducted by (Eriksson et al., 2010a), in which no effect on CH_4 production was observed as a result of simulated S deposition.

5.2.3. Effects of interactions between simultaneous warming and increased N and S depositions (Papers III, IV)

Ecosystems are often subjected to multiple simultaneously occurring environmental perturbations, where the effects of any individual perturbation are likely modulated by its interactions with the others. In this section, the hypothesis that the response of microbial communities to warming (described in Section 5.2) would be affected by increased N and/or S deposition is assessed.

Interactions between perturbations that amplified the effects of the individual perturbations on microbial community composition were observed, particularly in the upper depth horizons (Figure 1 in Paper III). The dissimilarity among communities increased with multiple perturbations, and the new community composition was not representative of the additive composition observed for individual perturbations (Figure 1 in Paper III). However, the community composition was generally similar for all treatment plots that received N addition (Figure 6), demonstrating that N is an important factor in strongly affecting, directly or indirectly, microbial communities in peatlands. This is most likely mainly an indirect effect occurring via induced changes in the plant community, as is discussed above (Section 5.2).

The specific functional genes that had a significant response to the perturbations at the AWTL horizon (see Section 5.2.1), reflected by either an increase or decrease in their relative abundance, were mainly a result of simultaneous perturbations. Generally, the effect of warming was modulated by simultaneous increased deposition of N and/ S (Figure 3A in Paper III). In particular for the marker genes that encode for methanogenesis, the effects of two-way interactions (NxS, NxT, SxT, where 'T' is 'temperature') were observed, counteracting the effect of individual perturbations and resulting in an increase in the relative abundance of such genes. On the contrary, the three-way interaction (NxSxT) further amplified the effects of the individual perturbations. At the BWTL horizon, however, few significant effects of the combined perturbations on the selected functional genes were observed (Figure 3B in Paper III).

Similarly, the abundance of *mcrA* transcript responded significantly to simultaneous perturbations, generally increasing with combined effects (Figure 1B in Paper IV). Such increases were the results of warming and/or increased S deposition in connection with simultaneous increase in N deposition (Figure 3B in Paper IV). Thereby, as found for the microbial community composition, N was the main factor

modulating the effect of the others. No effects of warming in combination with increased levels of S were observed, which contradicts the findings of Vile et al. (2003) and Gauci et al. (2004). However, Gauci et al. (2004) found that warming reduced the suppression of CH₄ emission by sulfates, while Vile et al. (2003) found the opposite.

5.3. Plant-microorganism interactions (Papers I, III, IV)

Correlations between the microbial and plant communities were found in all of the studies described in this thesis. At the in-situ experiment, the abundance of *mcrA* transcript was positively correlated with the relative abundance of the vascular plants *E. vaginatum*, *A. polifolia*, and *V. oxycoccus* (Paper IV). Similarly, a concordance between the plant and microbial taxonomic and functional composition was also observed (Paper III). Additionally, with regard to samples from the treatment plots that received additional NH₄NO₃, the microbial community composition was positively correlated with the relative abundance of the dominant vascular plants (*E. vaginatum*, *A. polifolia*, and *V. oxycoccus*) and negatively correlated with total *Sphagnum* spp. and *S. balticum* cover (Paper III). From the field study, a high degree of correspondence was found between both the archaeal and bacterial communities and the plant community among three peatlands (Paper I).

The analysis of co-occurrence networks between plants and microorganisms within three peatlands differing in climatological conditions and atmospheric depositions (Table 2 in Section 5.1) showed that each site had a specific network. They differed in terms of topological structures and species interactions, both AWTL and BWTL (Figure 4 and Table S4 in Paper I). Cena Mire had the most complex network structure (based on the topological indices), while Degerö Stormyr was the least complex. This indicates that the plant-microorganism networks in Cena Mire are more resilient than those of Dosenmoor and Degerö Stormyr, as the functions and resilience of such networks are reliant on their linkages (cf. Newman, 2003). The topological indices were positively related to the total N content in the pore water (Figure 6 in paper I), indicating that N availability in these N-limited ecosystems results in more complex and resilient networks. This may be due to the increase in vascular plants richness that likely resulted from higher N availability, which in turn may promote linkages with the microbial community. In contrast, the topological indices were disproportional to climate seasonality (TS/PS; Figure 6 in Paper I). The TS/PS ratio is supposed to have implications for plant growth, as some climates are expected to have strong interannual precipitation variations with lower temperature

amplitudes variation, or vice-versa (IPCC, 2013). High TS/PS values indicate that temperature seasonality is more variable than precipitation seasonality, while low TS/PS values indicate the opposite. The least resilient network was found in Degerö Stormyr, corresponding to the highest TS/PS value, while the most resilient network corresponded to the lowest TS/PS value. Thereby, interactions between species in peatlands in colder areas are more sensitive to climate change, and any shift in the interactions may impact ecosystem processes. These results are in line with the outcomes from the above results sections where increased N deposition followed by warming were found to be the two main factors affecting peat microbial communities.

Several keystone species – those that, in the event of their removal from the network, have a disproportionately deleterious effect on it – were identified and found to clearly contribute to the various network structures (Figure 4 in Paper I). The importance of plants as keystone species increased with increased network complexity, in line with a decrease in the importance of microorganisms (Figure 5b in Paper I). The plant community turnover among sites also contributed to network dissimilarities ($R^2_{adj} = 0.75$), while no significant contribution of the prokaryotes was observed (Figure 5a in Paper I). These results indicate that plants are the key drivers of interactions with microbial communities, which supports the general idea that plant communities have a direct effect on microbial community composition and activity (Fisk et al., 2003; Wardle et al., 2004; Wardle, 2006; Rooney-Varga et al., 2007; Robroek et al., 2015; Yavitt and Williams, 2015) and strengthens the argument put forth in the discussion above results sections: That the impact of temperature and N deposition on microbial communities is likely primarily indirect, occurring via induced changes in the plant community.

5.4. Summary of the results

Microbial community dynamics were influenced to a greater extent by large-scale than local-scale conditions. Overall, the turnover of microbial community composition along the field study sites correlated with both environmental conditions (temperature, precipitation, and N deposition) and nutrient content (Table 3). The three specifically investigated environmental perturbations (simulated warming and increased N and S depositions) caused substantial shifts in the composition of microbial communities, which were amplified by the interactive effects of the perturbations. The microbial community composition also correlated with vegetation community composition, as well as with CH₄ production rates.

Overall microbial functional potential was affected by the simulated N deposition and co-occurrence of increased S deposition and warming, and was correlated with vegetation community composition (Table 3). Interactions between plant and microbial communities were modulated by environmental parameters, where network complexity was weakened by interannual changes in temperature and increased with N content.

The abundance of active methanogens correlated with concentrations of DOC and NO_3^- in the pore water, the dominant vascular plant species, and CH_4 production rates (Table 3). A tendency towards a greater abundance of active methanogens resulting from higher temperatures and increased atmospheric N deposition was observed across the sites that differed with regard to these conditions, as well as the in-situ experiment (Table 3). Increased atmospheric N deposition amplified the effect of warming and increased S deposition on the active methanogens. The abundance of methanogens, investigated by qPCR analysis of the *mcrA* gene, and examination of the genes that encode for key steps in methanogenesis derived from the metagenome, revealed slightly different responses to the perturbations (Table 3). In both cases, (relative)abundance decreased with increased N deposition and the co-occurrence of the three perturbations, and increased with simultaneous warming and N or S deposition.

A recurrent issue in the work presented in this thesis was that the observed microbial responses demonstrated clear trends, but these were often not statistically significant. This is an intrinsic problem in complex ecological systems with many potential explanatory variable interactions. Microorganisms in the soil – the response variable in this case – also exhibit a high variability within a sample. Furthermore, ecological microbial data must often be analysed using non-parametric methods, which are more robust than parametric ones but lack in power. Taken together, these factors indicate that the trends observed and conclusions derived are not invalid, but must be cautiously interpreted.

Table 3. Summary of the results. N: Nitrogen deposition. T: Temperature. S: Sulfur deposition. P: Precipitation. DOC: Dissolved organic carbon. CH₄: Methane production rates. Red: Significant positive correlations. Blue: Significant negative correlations. Orange: Non-significant positive correlations. Green: Significant turnover of the microbial community composition. Purple: Correspondence between the microbial community and vegetation composition or CH₄ production rates. Grey: Non-significant results. White: Not applicable. The colour intensity (within each colour) denotes the strength of the correlation/response.

Microorganisms	Molecular marker	Molecular technique	Environmental perturbation							Nutrients	CH ₄	Plant community Composition
			N	T	S	P	NxS	NxT	SxT	NxSxT		
Microbial composition	16S rRNA	Amplicon sequencing										
Functional potential	Metagenome	Shotgun sequencing										
Active methanogens	<i>mcrA</i> transcripts	qPCR										
Bulk methanogens	<i>mcrA</i> gene	qPCR		*								
	Metagenome	Shotgun sequencing										
Diversity of methanogens	<i>mcrA</i> gene	T-RFLP		*								

*Soil temperature

6. Outcomes and reflections

The main aim of this thesis was to improve understanding of how microbial communities in peatlands respond to climate change and increased atmospheric N and S depositions. The results of the work show that geo-climatological conditions along the latitudinal gradient have a higher impact on microbial communities than the microhabitats within sites. Temperature and precipitation as well as atmospheric N and S depositions differed among the field study sites. However, these environmental factors are not perturbations per se, but ambient conditions at each site. Nevertheless, the fact that microbial community composition and abundance of active methanogens differed between the sites indicates the importance of these environmental factors in relation to microbial community dynamics. Both microbial community composition and active methanogens displayed a degree of variability among the site microhabitats, which can be attributed to peat biogeochemistry and plant composition (Figure 7). Thus, site-intrinsic spatial variability cannot be neglected when designing sampling strategies with the intention of obtaining a representative sample with which to study peat microbial communities and their effectors.

Of the three perturbations that were specifically investigated during the long-term in-situ experiment (warming and increased N and S depositions); N deposition followed by warming, played the most important role driving a shift of microbial community composition and promoting an increase in the abundance of active methanogens. It is hypothesised that this effect is mainly indirect, and occurs via an induced shift in vegetation towards vascular plants (Figure 7). This hypothesis is based on the clear shift in plant community composition in connection with warming and the addition of N, the strong relationship between plant and microbial communities that is mainly driven by the plant community, and the immediate uptake of nitrate by the plants following the addition of N. The impact of each perturbation on the microbial community was found to either multiply or counteract one another, with increased N deposition being the most important perturbation modulating the impact of warming and S deposition on the activity and composition of microbial communities. This highlights the importance of accounting for simultaneously occurring environmental perturbations in order to achieve better and more realistic projections of ecosystem responses to climate change. For example, precipitation regimes are projected to change and the results of this work indicate that will affect plant-microorganism interactions. While several studies have focused on dry/wet periods, future research should assess the interactive effects of

moisture regime dynamics with other perturbations, such as increased atmospheric N deposition and warming.

Figure 7. A schematic overview of the outcomes of this thesis. The environmental perturbations – nitrogen (N), temperature (T), precipitation (P), and sulfur (S) – and the nutrients that were found to individually have an impact on microbial communities are shown in coloured circles. A wider arrow implies a stronger observed effect. DOC: Dissolved organic carbon. DIN: Dissolved inorganic nitrogen.

Beyond environmental factors affecting the microbial community, plant community was found to play a key role in structuring microbial interactions. Plant-microbial interactions were in turn highly affected by N availability and seasonal fluctuations in temperature and precipitation. Given the fact that network complexity potentially determines the resistance and resilience of a network to perturbations, these results show that the linkage between above- and below-ground communities can either weaken and thereby alter ecosystem functioning, or strengthen thus ensuring ecosystem stability in the face of a changing climate. Long-term in-situ experiments simulating climatic perturbations would be the ideal method of elucidating the direction of the network response to a changing climate. This highlights the importance of accounting for the interactions between above- and below-ground

communities in order to better predict the response of an ecosystem to climate change.

Compositional analyses are useful tools for characterising microbial communities and their genetic content, allowing insights to be obtained regarding which taxa and functions of a microbial community correlate with ecosystem processes and overall biogeochemistry. The results of the work presented in this thesis show a large turnover in microbial taxonomic composition as a result of the investigated perturbations and their interactions. However, while multiple perturbations led to a large taxonomic shift, the changes that occurred in genome-encoded functional traits were relatively minor, indicating that the species selected for under each perturbation exhibit a substantial degree of functional redundancy. Thus, an important outcome of this thesis is that functional redundancy may be the defining factor in ecosystem resistance and resilience to long-term environmental change and constrain ecosystem services, such as GHG emissions and net C exchange between peatlands and the atmosphere.

In order to obtain more detailed insights of the implications of the microbial turnover with regard to ecosystem functioning, further investigations should focus on specific taxa that evinced a substantial response to the perturbations. Linking taxonomic composition to function is a challenge, and linking microbial community structure and ecosystem functioning even more so. There are several methods that provide a means of linking phylogeny with function. Among these, there are computational approaches for the taxonomic annotation of protein coding genes and transcripts or for their prediction from taxonomy data. There are also DNA- and RNA-stable isotope probing (SIP) methods that enable the identification of which taxa actively assimilate a priory labelled substrates. qPCR analyses of specific transcripts would complement the approach by enabling correlations between absolute abundance of expressed metabolic functions and process rates.

Overall, the microbial responses reported in this thesis fit the general framework of ecosystem processes in relation to the geo-climatic and microclimatic factors that has been developed by previous studies. However, a holistic view is required in order to reach a more complete understanding of ecosystem responses to climate change. Future research should optimally integrate microbial responses, in-situ process rate measurements, take into account network interactions between above- and below-ground communities, and general peat biogeochemistry, under long-term conditions considering multiple simultaneous environmental perturbations.

Acknowledgements

My sincere gratitude to my supervisors Bo Svensson and Åsa Danielsson: Bosse, thanks for your generosity, encouragement and much valuable advice. You have always guided me towards the right direction so as to make this thesis see the light. It was an honour to have you accompanying me in this journey; and Åsa, for your valuable input and the opportunity to conduct the research presented in this thesis. I would also like to thank Per-Eric Lindgren for putting your trust in me when the PEATBOG project started, and for welcoming me to perform the molecular work at your lab.

I am thankful to all the PEATBOG project members, and especially to Nancy Dise and Bjorn Robroek. The ERA-net project within the European Union's 6th Framework Programme is acknowledged for financing the BiodivERsA-PEATBOG project. The Swedish Research Council FORMAS and VR are also acknowledged for finance support. I am also thankful to Nordic Environmental NUcleotide Network for financing 3 weeks of research stay at Helsinki University, and to FEMS for the research fellowship award that financed 3 months of research stay at Copenhagen University.

I want to thank all my co-authors, especially to Alexander Eiler, Mats Nilsson, Bjorn Robroek, and Vincent Jassey. Thanks for your kindness, dedication and help. Working with brilliant minds is always challenging and rewarding. I have learned a lot from all of you.

I wish to thank all past and present Ph.D. students for making the Department – Environmental Friendly. Special thanks to Sepehr, for your interest and fruitful discussions that lead me into the right path; to Siva for your friendship and artistic skills; and to Luka for bringing the microbial spirit. I would also like to thank Lena Lundman, for your friendship and wise words, I am looking forward to the new battles the future holds for me.

Many thanks to Peter, Johanna, Fredrik, Henrik, Bella, Vaclav, Hanna, Rico, Matheus, Zineb, Behzad, and Razi, for making Linköping an enjoyable and vivid place. Johanna, thanks once again for help with the figures.

Thanks to all my friends from back home, to Clara, Joana, Quim, Núria, Júlia, Milena, Mireia... Gràcies per ser-hi!

Johan, tusen tack for your patience and support that have always given me confidence, comfort and encouragement. This thesis would have not been achieved without you. I also wish to thank my Swedish family, Anki, Lars and Marcus for making me feel like home.

Finalment, vull agrair tota la meva família per ser la millor família que un podria tenir. Especialment els meus pares, Sebastià i Mercè, pel vostre amor i suport incondicional. Sou el meu exemple a seguir. I a tu Sigrid, per ser el millor que m'ha dat la vida. Aquesta tesi és tan vostra com meva.

References

- Abram, J.W., Nedwell, D.B., 1978. Inhibition of methanogenesis by sulfate reducing bacteria competing for transferred hydrogen. *Archives of Microbiology* 117, 89-92.
- Andersen, R., Chapman, S.J., Artz, R.R.E., 2013. Microbial communities in natural and disturbed peatlands: A review. *Soil Biology and Biochemistry* 57, 979-994.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26, 32-46.
- Armstrong, W., Justin, S., Beckett, P.M., Lythe, S., 1991. Root adaptation to soil waterlogging. *Aquatic Botany* 39, 57-73.
- Artz, R.R.E., 2009. Microbial community structure and carbon substrate use in Northern peatlands. *Geophysical Monograph* 184, 111-129.
- Bergman, L., Svensson, B.H., Nilsson, M.B., 1998. Regulation of methane production in a Swedish acid mire by pH, temperature, and substrate. *Soil Biology and Biochemistry* 30, 729-741.
- Berry, D., Widder, S., 2014. Deciphering microbial interactions and detecting keystone species with co-occurrence networks. *Front Microbiol* 5, 219.
- Blake, L.I., Tveit, A., Ovreas, L., Head, I.M., Gray, N.D., 2015. Response of Methanogens in Arctic Sediments to Temperature and Methanogenic Substrate Availability. *PloS One* 10, e0129733.
- Blazewicz, S.J., Barnard, R.L., Daly, R.A., Firestone, M.K., 2013. Evaluating rRNA as an indicator of microbial activity in environmental communities: limitations and uses. *ISME J* 7, 2061-2068.
- Boot, C.M., Hall, E.K., Deneff, K., Baron, J.S., 2016. Long-term reactive nitrogen loading alters soil carbon and microbial community properties in a subalpine forest ecosystem. *Soil Biology & Biochemistry* 92, 211-220.
- Bragazza, L., Freeman, C., Jones, T., Rydin, H., Limpens, J., Fenner, N., . . . Toberman, H., 2006. Atmospheric nitrogen deposition promotes carbon loss from peat bogs. *Proceedings of the National Academy of Sciences of the United States of America* 103, 19386-19389.
- Bragazza, L., Buttler, A., Habermacher, J., Brancaleoni, L., Gerdol, R., Fritze, H., . . . Johnson, D., 2012. High nitrogen deposition alters the decomposition of bog plant litter and reduces carbon accumulation. *Global Change Biology* 18, 1163-1172.

- Bragazza, L., Parisod, J., Buttler, A., Bardgett, R.D., 2013. Biogeochemical plant-soil microbe feedback in response to climate warming in peatlands. *Nature Climate Change* 3, 273-277.
- Bragazza, L., Bardgett, R.D., Mitchell, E.A., Buttler, A., 2015. Linking soil microbial communities to vascular plant abundance along a climate gradient. *New Phytol* 205, 1175-1182.
- Bridgman, 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.
- Bubier, J.L., Moore, T.R., Bledzki, L.A., 2007. Effects of nutrient addition on vegetation and carbon cycling in an ombrotrophic bog. *Global Change Biology* 13, 1168-1186.
- Carvalhais, L.C., Dennis, P.G., Tyson, G.W., Schenk, P.M., 2012. Application of metatranscriptomics to soil environments. *Journal of Microbiological Methods* 91, 246-251.
- Castro, H.F., Classen, A.T., Austin, E.E., Norby, R.J., Schadt, C.W., 2010. Soil microbial community responses to multiple experimental climate change drivers. *Applied and Environmental Microbiology* 76, 999-1007.
- Chubukov, V., Gerosa, L., Kochanowski, K., Sauer, U., 2014. Coordination of microbial metabolism. *Nat Rev Microbiol* 12, 327-340.
- Clymo, R.S., 1963. Ion exchange in *Sphagnum* and its relation to bog ecology. *Annals of Botany* 27, 309-&.
- Clymo, R.S., Hayward, P.M., 1982. The ecology of *Sphagnum*. In: Smith, A.J.E. (Ed.), *Bryophyte ecology*. Chapman & Hall, London, pp. 229-289.
- Conrad, R., 1996. Soil microorganisms as controllers of atmospheric trace gases (H₂, CO, CH₄, OCS, N₂O, and NO). *Microbiological Reviews* 60, 609-640.
- Conrad, R., 1999. Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. *FEMS Microbiology Ecology* 28, 193-202.
- Contosta, A.R., Frey, S.D., Cooper, A.B., 2015. Soil microbial communities vary as much over time as with chronic warming and nitrogen additions. *Soil Biology and Biochemistry* 88, 19-24.

Crecchio, C., Stotzky, G., 1998. Binding of DNA on humic acids: effect on transformation of *Bacillus subtilis* and resistance to DNase. *Soil Biology & Biochemistry* 30, 1061-1067.

Cui, M., Ma, A., Qi, H., Zhuang, X., Zhuang, G., 2015. Anaerobic oxidation of methane: an "active" microbial process. *Microbiologyopen* 4, 1-11.

Currey, P.M., Johnson, D., Sheppard, L.J., Leith, I.D., Toberman, H., van der Wal, R., . . . Artz, R.R.E., 2010. Turnover of labile and recalcitrant soil carbon differ in response to nitrate and ammonium deposition in an ombrotrophic peatland. *Global Change Biology* 16, 2307-2321.

Deppenmeier, U., 2002. The unique biochemistry of methanogenesis. *Progress in Nucleic Acid Research and Molecular Biology* 71, 223-283.

Drake, H.L., Horn, M.A., Wust, P.K., 2009. Intermediary ecosystem metabolism as a main driver of methanogenesis in acidic wetland soil. *Environ Microbiol Rep* 1, 307-318.

Dunbar, J., Ticknor, L.O., Kuske, C.R., 2001. Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. *Applied and Environmental Microbiology* 67, 190-197.

Eriksson, T., Öquist, M.G., Nilsson, M.B., 2010a. Production and oxidation of methane in a boreal mire after a decade of increased temperature and nitrogen and sulfur deposition. *Global Change Biology* 16, 2130-2144.

Eriksson, T., Öquist, M.G., Nilsson, M.B., 2010b. Effects of decadal deposition of nitrogen and sulfur, and increased temperature, on methane emissions from a boreal peatland. *Journal of Geophysical Research* 115, 1-13.

Falkowski, P.G., Fenchel, T., Delong, E.F., 2008. The Microbial Engines That Drive Earth's Biogeochemical Cycles. *Science* 320, 1034-1039.

Fenner, N., Freeman, C., Maurice, A., Harmens, H., Reynolds, B., Sparks, T., 2007. Interactions between elevated CO₂ and warming could amplify DOC exports from peatland catchments. *Environmental Science & Technology* 41, 3146-3152.

Ferry, J.G., 2002. Methanogenesis Biochemistry. *Encyclopedia of life sciences* Nature Publishing Group, 1-9.

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America* 103, 626-631.

- Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME J* 6, 1007-1017.
- Fisk, M.C., Ruether, K.F., Yavitt, J.B., 2003. Microbial activity and functional composition among northern peatland ecosystems. *Soil Biology and Biochemistry* 35, 591-602.
- Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D.W., . . . Van Dorland, R., 2007. Changes in atmospheric constituents and in radiative forcing, Climate change 2007: The physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom, Cambridge University Press pp. 129-234.
- Freeman, L.C., 1977. A Set of Measures of Centrality Based on Betweenness. *Sociometry* 40, 35-41.
- Freitag, T.E., Prosser, J.I., 2009. Correlation of methane production and functional gene transcriptional activity in a peat soil. *Applied and Environmental Microbiology* 75, 6679-6687.
- Frolking, S., Roulet, N., Moore, T., Richard, P.J.H., Lavoie, M., Muller, S.D., 2001. Modeling Northern Peatland Decomposition and Peat Accumulation. *Ecosystems* 4, 479-498.
- Frolking, S., Roulet, N.T., 2007. Holocene radiative forcing impact of northern peatland carbon accumulation and methane emissions. *Global Change Biology* 13, 1079-1088.
- Galand, P.E., Fritze, H., Yrjala, K., 2003. Microsite-dependent changes in methanogenic populations in a boreal oligotrophic fen. *Environ Microbiol* 5, 1133-1143.
- Galand, P.E., Fritze, H., Conrad, R., Yrjala, K., 2005. Pathways for methanogenesis and diversity of methanogenic archaea in three boreal peatland ecosystems. *Applied and Environmental Microbiology* 71, 2195-2198.
- Galand, P.E., Yrjälä, K., Conrad, R., 2010. Stable carbon isotope fractionation during methanogenesis in three boreal peatland ecosystems. *Biogeosciences* 7, 3893-3900.
- Galloway, J.N., Aber, J.D., Erisman, J.W., Speitzinger, S.P., Howarth, R.W., Cowling, E.B., Cosby, B.J., 2003. The nitrogen cascade. *Bioscience* 53, 341-356.

- Galloway, J.N., Dentener, F.J., D.G., C., Boyer, E.W., Howarth, P.A., S.P., S., . . . Vörösmarty, C.J., 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry* 70, 153-226.
- Gans, J., Wolinsky, M., Dunbar, J., 2005. Computation improvements reveal great bacterial diversity and high metal toxicity in soil. 309 1387-1390.
- Garcia, J., Patel, B.K., Ollivier, B., 2000. Taxonomic, phylogenetic, and Ecological diversity of methanogenic *Archaea*. *Anaerobe* 6, 205-226.
- Gauci, V., Dise, N., 2002. Controls on suppression of methane flux from a peat bog subjected to simulated acid rain sulfate deposition. *Global Biogeochemical Cycles* 16, 1004.
- Gauci, V., Matthews, E., Dise, N., Walter, B., Koch, D., Granberg, G., Vile, M., 2004. Sulfur pollution suppression of the wetland methane source in the 20th and 21st centuries. *Proceedings of the National Academy of Science* 101, 12583-12587.
- Gorham, E., 1991. Northern peatlands: role in the carbon cycle and probable responses to climatic warming. *Ecological Applications* 1, 182-195.
- Graham, E.B., Knelman, J.E., Schindlbacher, A., Siciliano, S., Breulmann, M., Yannarell, A., . . . Nemergut, D.R., 2016. Microbes as engines of ecosystem function: when does community structure enhance predictions of ecosystem processes? *Front Microbiol* 7, 214.
- Granberg, G., Grip, H., Löfvenius, M.O., Sundh, I., Svensson, B.H., Nilsson, M., 1999. A simple model for simulation of water content, soil frost, and soil temperatures in boreal mixed mires. *Water Resources Research* 35, 3771-3782.
- Granberg, G., Sundh, I., Svensson, B.H., Nilsson, M., 2001. Effects of temperature, and nitrogen and sulfur deposition, on methane emission from a boreal mire. *Ecology* 82, 1982-1998.
- Griffiths, B.S., Philippot, L., 2013. Insights into the resistance and resilience of the soil microbial community. *FEMS Microbiology Reviews* 37, 112-129.
- Hamberger, A., Horn, M.A., Dumont, M.G., Murrell, J.C., Drake, H.L., 2008. Anaerobic consumers of monosaccharides in a moderately acidic fen. *Applied and Environmental Microbiology* 74, 3112-3120.
- Handelsman, J., 2004. Metagenomics: application of genomics to uncultured microorganisms. *Microbiology and Molecular Biology Reviews* 68, 669-685.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25, 1965-1978.

Holden, J., 2005. Peatland hydrology and carbon release: why small-scale process matters. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 363, 2891-2913.

Horn, M.A., Matthies, C., Kusel, K., Schramm, A., Drake, H.L., 2003. Hydrogenotrophic Methanogenesis by Moderately Acid-Tolerant Methanogens of a Methane-Emitting Acidic Peat. *Applied and Environmental Microbiology* 69, 74-83.

Hu, Y.-L., Jung, K., Zeng, D.-H., Chang, S.X., 2013. Nitrogen- and sulfur-deposition-altered soil microbial community functions and enzyme activities in a boreal mixedwood forest in western Canada. *Canadian Journal of Forest Research* 43, 777-784.

Hunger, S., Gossner, A.S., Drake, H.L., 2015. Anaerobic trophic interactions of contrasting methane-emitting mire soils: processes versus taxa. *FEMS Microbiol Ecol* 91.

IPCC, 2013. Climate change 2013: The physical science basic, working group I contribution to the IPCC fifth assessment report.

Jassey, V.E., Chiapusio, G., Binet, P., Buttler, A., Laggoun-Defarge, F., Delarue, F., . . . Gilbert, D., 2013. Above- and belowground linkages in Sphagnum peatland: climate warming affects plant-microbial interactions. *Global Change Biology* 19, 811-823.

Jassey, V.E.J., Lamentowicz, Ł., Robroek, B.J.M., Gąbka, M., Rusińska, A., Lamentowicz, M., De Deyn, G., 2014. Plant functional diversity drives niche-size-structure of dominant microbial consumers along a poor to extremely rich fen gradient. *Journal of Ecology* 102, 1150-1162.

Joabsson, A., Christensen, T.R., Wallen, B., 1999. Vascular plant controls on methane emissions from northern peatforming wetlands. *Trends in Ecology and Evolution* 14, 385-388.

Juottonen, H., Galand, P.E., Tuittila, E.-S., Laine, J., Fritze, H., Yrjala, K., 2005. Methanogen communities and Bacteria along an ecohydrological gradient in a northern raised bog complex. *Environ Microbiol* 7, 1547-1557.

Juottonen, H., Kotiaho, M., Robinson, D., Merila, P., Fritze, H., Tuittila, E.S., 2015. Microform-related community patterns of methane-cycling microbes in boreal Sphagnum bogs are site specific. *FEMS Microbiol Ecol* 91, fiv094.

Juottonen, H., Eiler, A., Biasi, C., Tuittila, E.S., Yrjala, K., Fritze, H., 2017. Distinct Anaerobic Bacterial Consumers of Cellobiose-Derived Carbon in Boreal Fens with Different CO₂/CH₄ Production Ratios. *Applied and Environmental Microbiology* 83.

Knief, C., 2014. Analysis of plant microbe interactions in the era of next generation sequencing technologies. *Front Plant Sci* 5, 216.

Kotiaho, M., Fritze, H., Merilä, P., Tuomivirta, T., Välimäki, M., Korhola, A., . . . Tuittila, E.-S., 2012. Actinobacteria community structure in the peat profile of boreal bogs follows a variation in the microtopographical gradient similar to vegetation. *Plant and Soil* 369, 103-114.

Kotsyurbenko, O.R., Chin, K.J., Glagolev, M.V., Stubner, S., Simankova, M.V., Nozhevnikova, A.N., Conrad, R., 2004. Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an acidic West-Siberian peat bog. *Environ Microbiol* 6, 1159-1173.

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 H₂-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.

Kristjansson, J.K., Schönheit, P., Thauer, R.K., 1982. Different K_s values for hydrogen of methanogenic bacteria and sulfate reducing bacteria – an explanation for the apparent inhibition of methanogenesis by sulfate. *Archives of Microbiology*, 131, 278-282.

Kupier, J.J., Mooij, W.M., Bragazza, L., Robroek, B.J.M., 2014. Plant functional types define magnitude of drought response in peatland CO₂ exchange. *Ecology* 95, 123-131.

Lai, D.Y.F., 2009. Methane Dynamics in Northern Peatlands: A Review. *Pedosphere* 19, 409-421.

Lamarque, J.F., Kiehl, J.T., Basseur, G.P., Butler, T., Cameron-Smith, P., Collins, W.D., . . . Thornton, P., 2005. Assessing future nitrogen deposition and carbon cycle feedback using a multimodel approach: Analysis of nitrogen deposition. *Journal of Geophysical Research* 110, D19303.

Landesman, W.J., Nelson, D.M., Fitzpatrick, M.C., 2014. Soil properties and tree species drive β -diversity of soil bacterial communities. *Soil Biology and Biochemistry* 76, 201-209.

Lawton, J.H., Brown, V.K., 1993. Redundancy in ecosystems, In: Schulze, E.D., Mooney, H.A. (Eds.), *Biodiversity and Ecosystem Function*. Springer-Verlag Berlin, Germany, pp. 255-270.

Leff, J.W., Jones, S.E., Prober, S.M., Barberan, A., Borer, E.T., Firn, J.L., . . . Fierer, N., 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences of the United States of America* 112, 10967-10972.

Legendre, P., Anderson, M.J., 1999. Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs* 69, 1-24.

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

Limpens, J., Granath, G., Gunnarsson, U., Aerts, R., Bayley, S., Bragazza, L., . . . Xu, B., 2011. Climatic modifiers of the response to nitrogen deposition in peat-forming *Sphagnum* mosses: a meta-analysis. *New Phytol* 191, 496-507.

Lin, X., Tfaily, M.M., Green, S.J., Steinweg, J.M., Chanton, P., Invittaya, A., . . . Kostka, J.E., 2014. Microbial metabolic potential for carbon degradation and nutrient (nitrogen and phosphorus) acquisition in an ombrotrophic peatland. *Applied and Environmental Microbiology* 80, 3531-3540.

Liu, D., Ding, W., Jia, Z., Cai, Z., 2012. The impact of dissolved organic carbon on the spatial variability of methanogenic archaea communities in natural wetland ecosystems across China. *Applied Microbiology and Biotechnology* 96, 253-263.

Liu, Y., Whitman, W.B., 2008. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *New York Academy of Sciences* 1125, 171-189.

Luo, Y., Weng, E., 2011. Dynamic disequilibrium of the terrestrial carbon cycle under global change. *Trends Ecol Evol* 26, 96-104.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27, 209-220.

Margulies, M., Egholm, M., Altman, W.E., Attiya, S., Bader, J.S., Bemben, L.A., . . . Rothberg, J.M., 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 437, 376-380.

- McCalley, C.K., Woodcroft, B.J., Hodgkins, S.B., Wehr, R.A., Kim, E.-H., Mondav, R., . . . Saleska, S.R., 2014. Methane dynamics regulated by microbial community response to permafrost thaw. *Nature* 514, 478-481.
- Medini, D., Donati, C., Tettelin, H., Masignani, V., Rappuoli, R., 2005. The microbial pan-genome. *Current Opinion in Genetics and Development* 15, 589-594.
- Minchin, P.R., Peter, R., 1987. An evaluation of relative robustness of techniques for ecological ordinations. *Vegetatio* 69, 89-107.
- Newman, M.E.J., 2003. The structure and function of complex networks. *SIAM* 42, 167-256.
- Nilsson, M., Sagerfors, J., Buffam, I., Laudon, H., Eriksson, T., Grelle, A., . . . Lindroth, A., 2008. Contemporary carbon accumulation in a boreal oligotrophic minerogenic mire - a significant sink after accounting for all C-fluxes. *Global Change Biology* 14, 2317-2332.
- Nilsson, M., Öquist, M., 2009. Partitioning Litter Mass Loss Into Carbon Dioxide and Methane in Peatland Ecosystems. *Geophysical Monograph* 184, 131-144.
- Olid, C., Bindler, R., Nilsson, M.B., Eriksson, T., Klaminder, J., 2017. Effects of warming and increased nitrogen and sulfur deposition on boreal mire geochemistry. *Applied Geochemistry* 78, 149-157.
- Peltionemi, K., Laiho, R., Juottonen, H., Bodrossy, L., Kell, D.K., Minkinen, K., . . . Fritze, H., 2016. Responses of methanogenic and methanotrophic communities to warming in varying moisture regimes of two boreal fens. *Soil Biology & Biochemistry* 97, 144-156.
- Peres-Neto, P., Jackson, D., 2001. How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. *Oecologia* 129, 169-178.
- Philippot, L., Ritz, K., Pandard, P., Hallin, S., Martin-Laurent, F., 2012. Standardisation of methods in soil microbiology: progress and challenges. *FEMS Microbiol Ecol* 82, 1-10.
- Phoenix, G.K., Emmett, B.A., Britton, A.J., Caporn, S.J.M., Dise, N.B., Helliwell, R., . . . Power, S.A., 2012. Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. *Global Change Biology* 18, 1197-1215.
- Poisot, T., Canard, E., Mouillot, D., Mouquet, N., Gravel, D., 2012. The dissimilarity of species interaction networks. *Ecol Lett* 15, 1353-1361.

Powell, J.R., Welsh, A., Hallin, S., 2015. Microbial functional diversity enhances predictive models linking environmental parameters to ecosystem properties. *Ecology* 96, 1985-1993.

Preston, M.D., Smemo, K.A., McLaughlin, J.W., Basiliko, N., 2012. Peatland microbial communities and decomposition processes in the James Bay lowlands, Canada. *Front Microbiol* 3, 70.

Prosser, J.I., Bohannan, B.J.M., Curtis, T.P., Ellis, R.J., Firestone, M.K., Freckleton, R.P., . . . Young, J.P.W., 2007. The role of ecological theory in microbial ecology. *Nature Reviews Microbiology* 5, 384-392.

Prosser, J.I., 2015. Dispersing misconceptions and identifying opportunities for the use of 'omics' in soil microbial ecology. *Nat Rev Microbiol* 13, 439-446.

Ramette, A., Tiedje, J.M., 2007. Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. *Proceedings of the National Academy of Sciences of the United States of America* 104, 2761-2766.

Ramirez, K.S., Lauber, C.L., Knight, R., Bradford, M.A., Fierer, N., 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. *Ecology* 91, 3463-3470.

Robroek, B.J.M., Schouten, M.G.C., Limpens, J., Berendse, F., Poorter, H., 2009. Interactive effects of water table and precipitation on net CO₂ assimilation of three co-occurring *Sphagnum* mosses differing in distribution above the water table. *Global Change Biology* 15, 680-691.

Robroek, B.J.M., Wubs, J.E.R., Martí, M., Zając, K., Palsgaard Andersen, J., Andersson, A., . . . Verhoeven, J.T.A., 2014. Microclimatological consequences for plant and microbial composition in *Sphagnum*-dominated peatlands. *Boreal Environ. Res.* 19, 195-208.

Robroek, B.J.M., Jassey, V.E.J., Kox, M.A.R., Berendsen, R.L., Mills, R.T.E., Cécillon, L., . . . Wurzbarger, N., 2015. Peatland vascular plant functional types affect methane dynamics by altering microbial community structure. *Journal of Ecology* 103, 925-934.

Robroek, B.J.M., Jassey, V.E., Martí, M., Bragazza, L., Bleeker, A., Buttler, A., . . . Verhoeven, J.T.A., In review. Taxonomic and functional turnover are decoupled in European peat bogs. *Nature Communications* In review.

Roling, W.F., 2007. Do microbial numbers count? Quantifying the regulation of biogeochemical fluxes by population size and cellular activity. *FEMS Microbiol Ecol* 62, 202-210.

- 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 Microbiol Ecol* 60, 240-251.
- Roulet, N.T., Lafleur, P.M., Richard, P.J.H., Moore, T.R., Humphreys, E.R., Bubier, J., 2007. Contemporary carbon balance and late Holocene carbon accumulation in a northern peatland. *Global Change Biology* 13, 397-411.
- Rousk, J., Bengtson, P., 2014. Microbial regulation of global biogeochemical cycles. *Frontiers in microbiology* 5, 1-3.
- Rydin, H., 1986. Competition and niche separation in *Sphagnum*. *Canadian Journal of Botany* 64, 1817-1824.
- Rydin, H., Jeglum, J.K., 2006. *The Biology of Peatlands*. Oxford University Press.
- Saarnio, S., Alm, J., Silvola, J., Lohila, A., Nykänen, H., Martikainen, P.J., 1997. Seasonal variation in CH₄ emissions and production and oxidation potentials at microsites on an oligotrophic pine fen. *Oecologia* 1997, 414-422.
- Schink, B., 1997. Energetics of syntrophic cooperation in methanogenic degradation. *Microbiology and Molecular Biology Reviews* 61, 262-280.
- Schmidt, O., Horn, M.A., Kolb, S., Drake, H.L., 2015. Temperature impacts differentially on the methanogenic food web of cellulose-supplemented peatland soil. *Environ Microbiol* 17, 720-734.
- Schmidt, O., Hink, L., Horn, M.A., Drake, H.L., 2016. Peat: home to novel syntrophic species that feed acetate- and hydrogen-scavenging methanogens. *ISME J* 10.
- Shen, R.-C., Xu, M., Chi, Y.-G., Yu, S., Wan, S.-Q., 2014. Soil Microbial Responses to Experimental Warming and Nitrogen Addition in a Temperate Steppe of Northern China. *Pedosphere* 24, 427-436.
- Shi, Y., Wang, Z., He, C., Zhang, X., Sheng, L., Ren, X., 2017. Using ¹³C isotopes to explore denitrification-dependent anaerobic methane oxidation in a paddy-peatland. *Scientific Reports* 7, 40848.
- Singh, B.K., Bardgett, R.D., Smith, P., Reay, D.S., 2010. Microorganisms and climate change: terrestrial feedbacks and mitigation options. *Nat Rev Microbiol* 8, 779-790.
- Smemo, K.A., Yavitt, J.B., 2007. Evidence for Anaerobic CH₄Oxidation in Freshwater Peatlands. *Geomicrobiology Journal* 24, 583-597.
- Smith, C.J., Osborn, A.M., 2009. Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology. *FEMS Microbiol Ecol* 67, 6-20.

Smith, S.J., van Aardenne, J., Klimont, Z., Andres, R.J., Volke, A., Delgado Arias, S., 2011. Anthropogenic sulfur dioxide emissions: 1850–2005. *Atmospheric Chemistry and Physics* 11, 1101-1116.

Staley, J.T., Konopka, A., 1985. Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annual Review of Microbiology* 39, 321-346.

Strickman, R.J., Fulthorpe, R.R., Coleman Wasik, J.K., Engstrom, D.R., Mitchell, C.P., 2016. Experimental sulfate amendment alters peatland bacterial community structure. *Science of the Total Environment* 566-567, 1289-1296.

Ström, L., Ekberg, A., Mastepanov, M., R., C.T., 2003. The effect of vascular plants on carbon turnover and methane emissions from a tundra wetland. *Global Change Biology* 9, 1185-1192.

Svensson, B.H., 1984. Different temperature optima for methane formation when enrichments from acid peat are supplemented with acetate or hydrogen. *Applied and Environmental Microbiology* 48, 389-394.

Svensson, B.H., Rosswall, T., 1984. In situ methane production from acid peat in plant communities with different moisture regimes in a subarctic mire. *Oikos* 43, 341-350.

Svensson, B.H., Sundh, I., 1992. Factors affecting methane production in peat soils. *Suo* 43, 1-8.

Tanaka, T., Kawasaki, K., Daimon, S., Kitagawa, W., Yamamoto, K., Tamaki, H., . . . Kamagata, Y., 2014. A hidden pitfall in the preparation of agar media undermines microorganism cultivability. *Applied and Environmental Microbiology* 80, 7659-7666.

Thomas, T., Gilbert, J., Meyer, F., 2012. Metagenomics - a guide from sampling to data analysis. *Microb Inform Exp* 2, 3.

Timmers, P.H., Welte, C.U., Koehorst, J.J., Plugge, C.M., Jetten, M.S., Stams, A.J., 2017. Reverse Methanogenesis and Respiration in Methanotrophic Archaea. *Archaea* 2017, 1654237.

Torsvik, V.L., Ovreas, L., Thingstad, T.F., 2002. Prokaryotic diversity: magnitude, dynamics, and controlling factors. *Science* 296, 1064-1066.

Turetsky, M.R., Treat, C.C., Waldrop, M.P., Waddington, J.M., Harden, J.W., McGuire, A.D., 2008. Short-term response of methane fluxes and methanogen activity to water table and soil warming manipulations in an Alaskan peatland. *Journal of Geophysical Research* 113, 1-15.

- Turetsky, M.R., Kotowska, A., Bubier, J., Dise, N.B., Crill, P., Hornibrook, E.R., . . . Wilkening, M., 2014. A synthesis of methane emissions from 71 northern, temperate, and subtropical wetlands. *Global Change Biology* 20, 2183-2197.
- Turunen, J., Tomppo, E., Tolonen, K., Reinikainen, A., 2002. Estimating carbon accumulation rates of undrained mires in Finland-application to boreal and subarctic regions. *The Holocene* 12, 69-80.
- Tveit, A., Schwacke, R., Svenning, M.M., Urich, T., 2013. Organic carbon transformations in high-Arctic peat soils: key functions and microorganisms. *ISME J* 7, 299-311.
- Tveit, A.T., Urich, T., Frenzel, P., Svenning, M.M., 2015. Metabolic and trophic interactions modulate methane production by Arctic peat microbiota in response to warming. *Proceedings of the National Academy of Sciences of the United States of America* 112, E2507-2516.
- Van Breemen, N., 1995. How Sphagnum bogs down other plants. *Trends Ecol Evol* 10, 270-275.
- Vaughan, D., Ord, B.G., 1982. An in vitro effect of soil organic matter fractions and synthetic humic acids on the generation of superoxide radicals. *Plant Soil* 66, 113-116.
- Vile, M.A., Bridgham, S.D., Wieder, R.K., Novák, M., 2003. Atmospheric sulfur deposition alters pathways of gaseous carbon production in peatlands. *Global Biogeochemical Cycles* 17, 27/21 - 27/27.
- Vincent, A.T., Derome, N., Boyle, B., Culley, A.I., Charette, S.J., 2016. Next-generation sequencing (NGS) in the microbiological world: How to make the most of your money. *Journal of Microbiological Methods*.
- Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. *Science* 304, 1629-1633.
- Wardle, D.A., 2006. The influence of biotic interactions on soil biodiversity. *Ecol Lett* 9, 870-886.
- Weedon, J.T., Kowalchuk, G.A., Aerts, R., Freriks, S., Roling, W.F., van Bodegom, P.M., 2017. Compositional Stability of the Bacterial Community in a Climate-Sensitive Sub-Arctic Peatland. *Front Microbiol* 8, 317.
- Whalen, S.C., 2005. Biogeochemistry of methane exchange between natural wetlands and the atmosphere. *Environmental Engineering Science* 22, 73-94.

- Widder, S., Allen, R.J., Pfeiffer, T., Curtis, T.P., Wiuf, C., Sloan, W.T., . . . Soyer, O.S., 2016. Challenges in microbial ecology: building predictive understanding of community function and dynamics. *ISME J* 10, 2557-2568.
- Wiedermann, M.N., Nordin, A., Gunnarsson, U., Nilsson, M.B., Ericsson, L., 2007. Global change shifts vegetation and plant-parasite interactions in a boreal mire. *Ecology* 88, 454-464.
- Williams, R.J., Howe, A., Hofmockel, K.S., 2014. Demonstrating microbial co-occurrence pattern analyses within and between ecosystems. *Front Microbiol* 5, 358.
- Wuebbles, D.J., Hayhoe, K., 2002. Atmospheric methane and global change. *Earth-Science Reviews* 57, 177-210.
- Wust, P.K., Horn, M.A., Drake, H.L., 2009. Trophic links between fermenters and methanogens in a moderately acidic fen soil. *Environ Microbiol* 11, 1395-1409.
- Xu, Y., Fan, J., Ding, W., Gunina, A., Chen, Z., Bol, R., . . . Bolan, N., 2017. Characterization of organic carbon in decomposing litter exposed to nitrogen and sulfur additions: Links to microbial community composition and activity. *Geoderma* 286, 116-124.
- Yavitt, J.B., Yashiro, E., Cadillo-Quiroz, H., Zinder, S.H., 2012. Methanogen diversity and community composition in peatlands of the central to northern Appalachian Mountain region, North America. *Biogeochemistry* 109, 117-131.
- Yavitt, J.B., Williams, C.J., 2015. Linking tree species identity to anaerobic microbial activity in a forested wetland soil via leaf litter decomposition and leaf carbon fractions. *Plant and Soil* 390, 293-305.
- Yu, Z.C., 2012. Northern peatland carbon stocks and dynamics: a review. *Biogeosciences* 9, 4071-4085.
- Zeng, J., Liu, X., Song, L., Lin, X., Zhang, H., Shen, C., Chu, H., 2016. Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. *Soil Biology and Biochemistry* 92, 41-49.
- Zhou, J., Guan, D., Zhou, B., Zhao, B., Ma, M., Qin, J., . . . Li, J., 2015. Influence of 34-years of fertilization on bacterial communities in an intensively cultivated black soil in northeast China. *Soil Biology & Biochemistry* 90, 42-51.

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