Aus der Professur für Landschaftsökologie und Standortkunde der Agrar- und Umweltwissenschaftlichen Fakultät

Microbial community dynamics and methane cycling in rewetted temperate fens

Dissertation

zur Erlangung des akademischen Grades

Doktor der Agrarwissenschaften (Dr. agr.)

an der Agrar- und Umweltwissenschaftlichen Fakultät der Universität Rostock

vorgelegt von

M. Sc. Viktoria R. Unger (Rostock)

Rostock, den 07.10.2020

https://doi.org/10.18453/rosdok_id00003627

Promotion

Microbial community dynamics and methane cycling in rewetted temperate fens

Grunddaten

Promovend	Viktoria R. Unger
Akademischer Grad	Dr. agr.
Promotionsgebiet	Landschaftsökologie und Standortkunde
Datum der Einreichung	07.10.2020
Datum der mündlichen Prüfungsleistung	16.12.2021

Betreuer/Gutachter

Dr. Gerald Jurasinski, Universität Rostock	Betreuer und Gutachter
Dr. Heli Juottonen, University of Jyväskylä / Finland	Gutachter
Prof. Dr. Lars Kutzbach, Universität Hamburg	Gutachter
Prof. Dr. Tim Urich, Universität Greifswald	Gutachter

Einrichtung: Agrar- und Umweltwissenschaftliche Fakultät

Summary

A current focal point in climate research is understanding how disturbances to peatland hydrology alter the cycling of methane, a potent greenhouse gas produced primarily by microbes of the domain archaea. In intact peatland ecosystems, high water table levels favor slower decomposition processes and foster soil organic matter accumulation. Consequently, peatlands store vast amounts of terrestrial organic carbon. However, much of the world's peatlands have been drained and utilized for agriculture, forestry, or peat mining. Drainage promotes rapid decomposition of the soil organic matter stock, and results in significant carbon dioxide emissions. Thus, despite covering just 0.3 % of the global land area, drained peatlands represent approximately 5 % of global anthropogenic greenhouse gas emissions. Currently, efforts are expanding to restore drained peatlands via rewetting. Rewetting involves permanent raising of the water table level, and is meant to reduce greenhouse gas emissions, and promote peat accumulation. Rewetting, however, can increase methane emissions dramatically, sometimes for up to several decades. High post-rewetting methane emissions have been measured even in coastal peatlands, where an abundance of sulfate is assumed to inhibit microbial methane production.

The factors that influence the magnitude and duration of increased methane emissions are not well-resolved. Water level alterations can induce changes in microbial communities both directly and indirectly involved in methane cycling, which can in turn alter their control over methane production and emission. Further, a biogeochemical legacy of drainage left in the form of polyphenol degradation could potentially enhance microbial carbon turnover, even after a peatland is rewetted. However, microbial community composition, abundances, and activity have rarely been investigated in rewetted peatlands in the context of methane cycling, especially in temperate fens. Studies of peat microbial communities would provide fundamental insight into observed patterns in methane emissions in rewetted fens. Such information could be valuable for land management decisions regarding rewetting projects and methane emission mitigation.

Besides anthropogenic water level manipulations, climate-change related extreme weather events such as drought are expected to increase in frequency and intensity. A better understanding of microbial community dynamics is essential for anticipating climate-change related alterations to hydrology, and ultimately methane emissions in rewetted fens. Therefore, in the following work, I focused on characterizing *in situ* peat microbial community composition and abundances in two rewetted temperate fens with high methane emissions, a coastal brackish fen and a freshwater riparian fen. I included microbial activity estimations to assess the importance of drainage-induced polyphenol degradation in promoting increased carbon turnover in rewetted fen peats. Finally, during a natural drought, I characterized the *in situ* microbial community dynamics underlying a dramatic decrease in ecosystem methane emissions. The four studies in this thesis provide fundamental insights into the microbial community dynamics of rewetted temperate fens, and represent novel contributions to a body of literature in which temperate fens are underrepresented.

Zusammenfassung

Ein aktueller Schwerpunkt in der Klimaforschung ist das Verständnis, wie Störungen in der Hydrologie von Mooren den Kreislauf von Methan verändern, einem potenten Treibhausgas, das vor allem von Mikroorganismen der Domäne Archaeen produziert wird. In intakten Moorökosystemen begünstigen hohe Wasserspiegel langsamere Zersetzungsprozesse und fördern die Akkumulation organischer Bodensubstanz. Folglich speichern Torfgebiete große Mengen an terrestrischem organischem Kohlenstoff. Ein Großteil der Moorgebiete der Welt wurde jedoch entwässert und für die Land- und Forstwirtschaft oder den Torfabbau genutzt. Die Dränage fördert den schnellen Abbau der organischen Bodensubstanz und führt zu erheblichen Kohlendioxidemissionen. Obwohl sie nur 0,3 % der globalen Landfläche bedecken, machen entwässerte Torfgebiete etwa 5 % der weltweiten anthropogenen Treibhausgasemissionen aus. Gegenwärtig werden die Bemühungen ausgeweitet, entwässerte Torfgebiete durch Wiedervernässung zu renaturieren. Die Wiedervernässung beinhaltet eine permanente Anhebung des Wasserspiegels, mit dem Ziel, Treibhausgasemissionen zu reduzieren und Torfanreicherung zu fördern. Die Wiedervernässung kann jedoch die Methanemissionen dramatisch erhöhen, manchmal über mehrere Jahrzehnte lang. Hohe Methanemissionen nach der Wiedervernässung wurden sogar in Küstenmooren gemessen, wo erwartungsgemäß die mikrobielle Methanproduktion durch einen Überschuss an Sulfat gehemmt wird.

Die Faktoren, die das Ausmaß und die Dauer der erhöhten Methanemissionen beeinflussen, sind nicht gut aufgeklärt. Veränderungen des Wasserspiegels können Veränderungen in mikrobiellen Gemeinschaften hervorrufen, die direkt oder indirekt am Methankreislauf beteiligt sind, was wiederum ihren Einfluss auf die Methanproduktion und -emission verändern kann. Darüber hinaus könnte ein biogeochemisches Relikt der Drainage in Form von Polyphenolabbau den mikrobiellen Kohlenstoffumsatz potenziell erhöhen, selbst wenn ein Moor wiedervernässt wird. Zusammensetzung, Abundanz und Aktivität der mikrobiellen Gemeinschaften sind in wiedervernässten Mooren im Zusammenhang mit dem Methankreislauf jedoch wenig erforscht, insbesondere in Niedermooren gemäßigter Klimazonen. Untersuchungen über mikrobielle Gemeinschaften im Torf würden einen grundlegenden Einblick in die beobachteten Muster der Methanemissionen in wiedervernässten Niedermooren geben. Solche Informationen können für Landmanagement-Entscheidungen über Wiedervernässungsprojekte und die Minderung von Methanemissionen wertvoll sein.

Neben den anthropogenen Wasserspiegelmanipulationen ist zu erwarten, dass durch den Klimawandel bedingte extreme Wetterereignisse wie Dürren an Häufigkeit und Intensität zunehmen werden. Ein besseres Verständnis der Dynamik mikrobieller Gemeinschaften ist für das Verständnis klimawandelbedingter Veränderungen der Hydrologie und letztlich der Methanemissionen in wiedervernässten Niedermooren unerlässlich. Daher konzentrierte ich mich in der folgenden Arbeit auf die Charakterisierung der *in-situ*-Zusammensetzung und Abundanzen mikrobieller Torfgemeinschaften in zwei wiedervernässten Niedermooren der gemäßigten Zonen mit hohen Methanemissionen, einem küstennahes Brackwassermoor und einem Süßwasser-Flußtal. Ich schloss Schätzungen der mikrobiellen Aktivität ein, um die Bedeutung des durch Entwässerung hervorgerufenen Polyphenolabbaus für die Förderung eines erhöhten Kohlenstoffumsatzes in wiedervernässten Niedermooren zu beurteilen. Schließlich charakterisierte ich die Dynamik der mikrobiellen Gemeinschaft *in situ*, die einem dramatischen Rückgang der Methanemissionen im Ökosystem während einer natürlichen Dürre zugrunde liegt. Die vier Studien in dieser Arbeit liefern grundlegende Einsichten in die Dynamik der mikrobiellen Gemeinschaft in wiedervernässten Niedermooren und stellen neuartige Beiträge zu einer Literatur dar, in der Niedermoore in gemäßigten Zonen unterrepräsentiert sind.

Contents

Appendix: Research articles in full text

List of Figures

List of Tables

List of Publications and Author Contributions

A. Wen, X., **Unger, V.**, Jurasinski, G., Koebsch, F., Horn, F., Rehder, G., Sachs, T., Zak, D., Lischeid, G., Knorr, K-H., Böttcher, M. E., Winkel, M., Bodelier, P. L. E., and Liebner, S. (2018). Predominance of methanogens over methanotrophs in rewetted fens characterized by high methane emissions. *Biogeosciences* 15: 6519-6536. DOI: 10.5194/bg-15-6519-2018

Unger, V.: Shared first authorship/corresponding author, original draft, study design, data analysis, lab work, editing

➢ *Appendix A of this thesis*

B. Koebsch, F., Winkel, M., Liebner, S., Liu, B., Westphal, J., Schmiedinger, I., Spitzy, A., Gehre, M., Jurasinski, G., Köhler, S., **Unger, V.**, Koch, M., Sachs, T., and Böttcher, M. E. (2019). Sulfate deprivation triggers high methane production in a disturbed and rewetted coastal peatland. *Biogeosciences* 16: 1937-1953. DOI: 10.5194/bg-16-1937-2019

Unger, V.: Lab work, editing

➢ *Appendix B of this thesis*

C. Zak, D., Roth, C., **Unger, V.**, Goldhammer, T., Fenner, N., Freeman, C., and Jurasinski, G. (2019). Unravelling the importance of polyphenols for microbial carbon mineralization in rewetted riparian peatlands. *Frontiers in Environmental Science* 7: 147. DOI: 10.3389/fenvs.2019.00147

Unger, V.: Writing, editing

➢ *Appendix C of this thesis*

D. **Unger, V.**, Liebner, S., Koebsch, F., Horn, F., Yang, S., Sachs, T., Kallmeyer, J., Knorr, K.-H., Rehder, G., Gottschalk, P., and Jurasinski, G. Congruent changes in microbial community dynamics and ecosystem methane fluxes following natural drought in two restored fens. submitted to *Soil Biology and Biochemistry.*

  Unger, V.: Corresponding author, original draft, study design, field work, lab work, data analysis, editing

  ➢ *Appendix D of this thesis*

1 Introduction

1.1 Background and motivation

Via primary production, peatland macrophytes convert large amounts of the greenhouse gas (GHG) carbon dioxde (CO_2) to plant biomass (i.e., organic matter), most of which is ultimately buried under waterlogged, anaerobic conditions. On one hand, anaerobic conditions foster slower decomposition, polyphenol accumulation, and therefore organic matter accumulation (Clymo, 1984; Freeman et al., 2001). On the other hand, they promote the production of methane (CH_4) – a GHG with a global warming potential 28 times stronger than CO_2 on a 100-year timescale (Myhre et al., 2013; Saunois et al., 2020). Consequently, peatlands play an important dual-role in the global carbon (C) cycle, as they store approximately 30 % of the world's terrestrial organic C (Mitsch & Gosselink, 2015), but are a significant source of atmospheric CH_4 (Saunois et al., 2020). Undisturbed peatlands are typically a net C sink; however, most peatlands have been subject to anthropogenic water level manipulations (Joosten, 2009), and the CH_4 cycle in these systems, as well as the involved microbial communities, seem to be highly vulnerable to hydrological disturbances (Vanselow-Algan et al., 2015; Wen and Unger et al., 2018).

Globally, vast areas of peatlands have been drained and converted to farmland, pasture, or harvested for peat (Joosten, 2009). Since aerobic decomposition of accumulated organic matter may proceed under drained conditions, drained peatlands are a significant source of CO_2. From 1990 to 2008, global CO_2 emissions from drained peatlands increased by 20 % (Joosten, 2009). There are now widespread attempts to restore these ecosystems via rewetting (i.e., permanently raising water table level) – especially in Europe – the world's second largest source of GHGs associated with peatland drainage as of 2009 (Joosten, 2009). While rewetting reduces CO_2 emissions (Wilson et al., 2016), it can increase CH_4 emissions significantly (Hahn et al., 2015), sometimes for at least several decades (Vanselow-Algan et al., 2015). This pulse in CH_4 emissions is assumed to decrease and become negligible after several decades, and recent research suggests rewetting projects should be implemented regardless of potential increases in CH_4 emissions (Günther et al., 2020). Nevertheless, rewetting projects should ideally be implemented with the goal of minimizing the magnitude and duration of CH_4 release. Fens (base-rich, groundwater-fed peatlands) are the most ubiquitous peatland type in temperate regions (Emsens et al., 2020), and have some of the highest recorded post-rewetting CH_4

emissions (Abdalla et al., 2016; Hahn et al., 2015), but, compared to bogs (acidic, precipitation-fed peatlands) have received little attention (Cadillo-Quiroz et al., 2008; Emsens et al., 2020). A handful of studies examining microbial communities and methane cycling in rewetted fens have been conducted in oligotrophic and mesotrophic boreal fens (e.g., Juottonen et al., 2005; 2012), alpine fens (e.g., Cheema et al., 2015; Franchini et al., 2015; Liebner et al., 2012; Urbanová et al., 2013) and subarctic fens (Liebner et al., 2015), but unlike minerotrophic temperate fens, post-rewetting CH_4 emissions in these systems were generally not significantly higher than in pristine sites. Furthermore, microbial communities in temperate fens are likely to respond differently to water level changes, and would therefore exhibit different controlling patterns over CH_4 cycling.

Due to high post-rewetting CH_4 emissions, and the uncertainty surrounding the duration of altered emissions, there is already an ugent need to characterize microbial communities involved in CH_4 cycling in temperate fens. However, in addition to anthropogenic water level alterations, future climate-change related alterations to fen hydrology (and therefore CH_4 cycling; IPCC, 2014) will add an additional challenge to constraining methane budgets in these systems. So far, a number of incubation studies have been conducted to analyze drought effects on CH_4-cycling microbes, but *in situ* studies of drought-affected CH_4-cylcing microbial communities are exceedingly scarce, and microbial communities may behave differently *in situ*.

1.2 Methane biogeochemistry in peatlands: importance of water level

Plant community composition, site geochemistry, nutrient status, and microbial community dynamics all influence peatland CH_4 emissions, but of particular importance is water level (Abdalla et al., 2016; Mitsch & Gosselink, 2015; Serrano-Silva et al., 2014; Smith et al., 2018; Waddington & Day, 2007). In addition to directly influencing CH_4 production and emission, water level can affect the relative influence of other controlling factors (Waddington et al., 1996; Waddington & Day, 2007). A number of studies have demonstrated strong correlation between water level and CH_4 emissions in peatlands (Abdalla et al., 2016; Augustin et al., 1998; Kang et al., 2018; Moore & Roulet, 1993; Shao et al., 2017; Tuittila et al., 2000; Waddington et al., 1996). Raising water level near to or above the peat surface – especially after prolonged drainage – increases CH_4 emissions (Turetsky et al., 2014; Waddington & Day, 2007). A meta-analysis by Abdalla et al. (2016) found rewetting caused a 46 % average increase in CH_4 emissions for northern peatlands. With respect to water level changes, fens may be even more sensitive than bogs. Evidence for this has been found in studies of vegetation and microbial community dynamics, as well as C biogeochemistry (Jaatinen et al., 2007; Komulainen et al., 1999; Laine et al., 1995; Peltoniemi et al., 2015; Urbanová & Bárta, 2016).

Water level reduction (to below the peat surface) is typically associated with a decrease in CH_4 emissions (Smith et al., 2018; Turetsky et al., 2014). However, water level reduction can influence CH_4 cycling in a number of ways, depending on the duration of the reduction. Evidence is accumulating for a biogeochemical legacy of long-term drainage (Emsens et al., 2020; Freeman et al., 2001; Zak et al., 2019), with potentially lasting effects on peatland CH_4 cycling (McNicol et al., 2020). In undrained peatlands, anaerobic conditions lead to an accumulation of polyphenolic compounds which can inhibit microbial activity (including that of CH_4-producers), and therefore C mineralization. In bogs, this is thought to prevent large amounts of organic C from being released to the atmosphere (Freeman et al., 2001). Draining introduces molecular oxygen which activates phenol oxidase, the enzyme that catalyzes the breakdown of accumulated polyphenols. Long-term drainage can thus reduce peat polyphenol content, potentially permitting higher microbial C turnover (e.g. CH_4 production) even after the peatland is rewetted (Zak et al., 2019). There is some indirect evidence for this in fens, as significantly higher concentrations of dissolved (Zak & Gelbrecht, 2007) and total organic C (Hahn et al., 2015) have been measured in rewetted fens, together with a drastic increase in CH_4 emissions. Additionally, McNicol et al. (2020) found that approximately 30 % of the total CH_4 emissions in a rewetted Californian peatland could be attributed to old, degraded peat. The relative importance of polyphenols in different types of peatlands is currently under discussion, however, and has hardly been examined in fens (Pinsonneault et al., 2016; Zak et al., 2019). A reduction in peat polyphenol content could be one factor contributing to the high CH_4 emissions observed in rewetted fens.

Short-term water level reduction (i.e., several weeks to months) decreases CH_4 emissions directly by introducing oxygenated conditions, and can stimulate of reoxidation of alternative terminal electron acceptors (TEAs) utilized in the microbial breakdown of organic matter, such as sulfate (SO_4^{2-}), nitrate (NO_3^-), and metal oxides. These TEAs, which are energetically more favorable (Fig. 2-1), are preferentially reduced by microbes which are then able to outcompete CH_4-producing organisms for substrate (Dean et al., 2018). Thus, this mechanism can effectively inhibit methanogenesis even after water levels again increase, at least until the supply of alternative TEAs is depleted (Knorr & Blodau, 2009). Additionally, geochemical evidence from incubation studies suggests that short-term water level decreases (e.g., drought events) can stimulate CH_4 oxidation (Knorr et al., 2008) – a microbial process that can reduce CH_4 emissions from peatlands significantly (Yavitt et al., 1988).

Robust, efficient methods for characterizing microbial communities in the environment were developed largely in the last few decades (Rincon-Florez et al., 2013). So far, it is known that microbial communities both directly and indirectly involved in CH_4 cycling are themselves sensitive to water level changes, which can in turn influence their patterns of control over CH_4 cycling (Kim et al., 2008; Peltoniemi et al., 2016). A comprehensive understanding of the microbial community dynamics associated with changing water levels would provide further insight into observed changes in CH_4 cycling, and aid in anticipating future changes to CH_4 cycling in peatlands subject to drainage, rewetting and/or extreme weather events such as flooding or drought.

2 Microbial agents of the peatland CH_4 cycle

2.1 Microbial CH_4 transformation in peatlands

CH_4 emitted from peatlands is a byproduct of anaerobic microbial metabolism performed by organisms termed methanogens (Segers, 1998). Produced CH_4 may reach the atmosphere via diffusion, ebullition (i.e., bubbling), and/or direct transport through plant aerenchyma – specialized porous tissues that are common in aquatic plants (Abdalla et al., 2016; Baird et al., 2004; Joabsson et al., 1999; Segers, 1998). CH_4 that is not transported directly to the atmosphere via ebullition or plant aerenchyma can be oxidized by organisms known as methanotrophs, the majority of which are aerobic bacteria typically living along the oxic-anoxic peat interface (Krumholz et al., 1995). This process can reduce CH_4 emissions from peatlands significantly in intact peatlands (Yavitt et al., 1988). Indeed, aerobic methanotrophs represent a globally significant control on peatland CH_4 emissions (Conrad, 2007; Hanson and Hanson, 1996). While other factors influence CH_4 emission rates from peatlands, the emitted CH_4 is ultimately the result of the balance between CH_4 production and CH_4 consumption by these microorganisms.

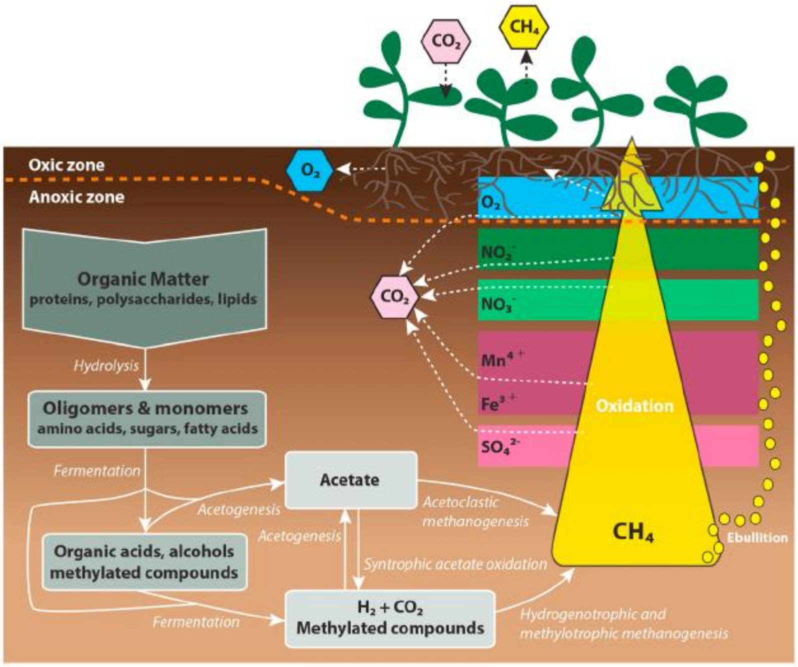


Figure 2-1 – Conceptual diagram of CH_4 production and consumption in peatlands. Microbially mediated processes are shown in italics. (from: Dean et al., 2018)

2.2 Methanogens

2.2.1 *Metabolism of CH_4 production*

In anaerobic peatlands, methanogenesis is the final step in organic matter decomposition, and occurs when other more energetically favorable electron acceptors (mainly O_2, NO_3^-, Fe^{3+}, Mn^{4+}, SO_4^{2-}) are depleted (Dean et al., 2018; Conrad 2020; Fig. 2-1). Three distinct pathways of methanogenesis are known: acetate cleavage (acetoclastic methanogenesis), CO_2 reduction with H_2 (hydrogenotrophic methanogenesis), and reduction of methylated compounds such as methanol, methyl-amines, and methyl-sulfides (methylotrophic methanogenesis; (Dziewit et al., 2015; Fenchel et al., 2012; Sikora et al., 2017). Methanogens are thus often categorized as acetoclastic, hydrogentrophic, or methylotrophic, based on substrate use. Regardless of substrate, the terminal step in methanogenesis is the same, and the reaction is catalyzed by the enzymatic complex methylcoenzyme M reductase (Mcr; Borrel et al., 2013). The *mcrA* gene that encodes the alpha subunit of Mcr is therefore present in all known methanogens, and is utilized in culture-independent analyses of methanogens in the environment (Sikora et al., 2017). Methanogens can survive under a wide range of extreme conditions, including acidic, alkaline and hypersaline environments, among others (Evans et al., 2019). In peatlands, CH_4 is produced primarily via acetoclastic and hydrogenotrophic methanogenesis. In fens, acetoclastic methanogenesis is typically the dominant CH_4 formation mechanism in the upper peat layers, with a shift toward hydrogenotrophic methanogenesis in the deeper layers (Galand et al., 2005; Wen and Unger et al., 2018).

2.2.2 *Current taxonomy of methanogens*

The long-held paradigm that methanogenesis is restricted to members of the domain archaea has been challenged in recent years (Bižić et al., 2020). Nevertheless, it is currently believed that peatland CH_4 production is performed primarily by methanogenic archaea. Initially, archaeal methanogens were classified into five orders in the phylum Euryarchaeota, including Methanosarcinales, Methanobacteriales, Methanomicrobiales, Methanopyrales, and Methanococcales (Luo et al., 2009). Culture-independent studies have since expanded our knowledge of methanogenic metabolic diversity (Evans et al., 2019), and provided metagenomic evidence for methanogenesis in the phyla Bathyarchaeota (now classified as Bathyarchaeia, within Crenarchaeota; Evans et al., 2015) and Verstraetearchaeota (Vanwonterghem et al., 2016). CH_4 production in peatlands is known to be performed by members of the orders Methanosarcinales, Methanomicrobiales, Methanobacteriales, Methanocellales, and Methanomassiliicoccales (Evans et al., 2019). Acetoclastic methanogenesis is thought to be performed exclusively by members of Methanosarcinales, whereas hydrogenotrophic methanogenesis is widespread among methanogenic orders (Luo et al., 2009). Methanotrichaceae (often called Methanosaetaceae), a family limited to acetoclastic methanogensis, is often detected in fens (Cadillo-Quiroz et al., 2008; Cheema et al., 2015), sometimes in relatively high abundance (Wen and Unger et al., 2018; Unger et al., *submitted*). Methylotrophic methanogens detected in fens includes the families Methanomassiliicoccaceae, Methanoregulaceae, and Methanocellaceae (Wen and Unger et al., 2018; Killham & Prosser, 2006).

2.3 Methanotrophs

2.3.1 *Aerobic CH_4 metabolism*

Aerobic methanotrophs utilize CH_4 as their sole C and energy source, ultimately oxidizing CH_4 to CO_2 (McDonald et al., 2008). The first step in this mechanism is catalyzed by CH_4 monooxygenase (MMO), which has both a particulate membrane-bound form (pMMO) and soluble cytoplasmic form (sMMO). Currently, all known aerobic methanotrophs besides the genera *Methylocella* and *Methyloferula* possess pMMO (Dedysh & Dunfield, 2016; Liebner & Svenning, 2013) while only several strains possess sMMO (Hanson and Hanson, 1996; McDonald et al., 2008). Subunits of pMMO and sMMO are encoded by the *pmoA* and *mmoX* genes, respectively, and as such, these genes are often employed in analyses of aerobic methanotrophic communities (Knief, 2015). Based on their metabolism, cell morphology, and phylogeny, aerobic methanotrophs are classified as type I, type II or type X (McDonald et al., 2008). Type I and X methanotrophs are known to use the ribulose monophosphate (RuMP) pathway for formaldehyde fixation, while type II methanotrophs utilize the serine pathway (Hanson and Hanson, 1996). Type X methanotrophs are distinct from type I methanotrophs because of other biochemical and phylogenetic differences, as well as their ability to grow at temperatures above 45ºC (Hanson and Hanson, 1996; Bowman, 2006; Knief, 2015). Recent incubation studies suggest respective subgroups may be ecologically distinct, possessing different traits according to their survival strategy (Ho et al., 2013a; Knief, 2015; Krause et al., 2014; van Kruistum et al., 2018).

2.3.2 *Current taxonomy of aerobic methanotrophs*

Aerobic CH_4 oxidizers are classified within the bacterial phyla Proteobacteria and Verrucomicrobia (Krause et al., 2015). Methanotrophs of the Verrucomicrobia were recently discovered in geothermal vents, and little is known about their potential distribution in other environments (Dedysh, 2009; Op den Camp et al., 2018). Members of the Gamma- and Alphaproteobacteria (comprising type I and type II methanotrophs, respectively), are the primary CH_4 oxidizers in peatlands (Bodelier et al., 2011). Gammaproteobacterial methanotrophs are classified within the order Methylococcales, and families Methylomonaceae, and Methylococcaceae. Alphaproteobacterial methanotrophs are classified within the order Rhizobiales and family Beijerinckiaceae (Smith & Wrighton, 2019). Type II methanotrophs of the Alphaproteobacteria often dominate the methanotroph community in *Sphagnum* peatlands (i.e., bogs). In more alkaline peatlands (i.e., fens) Gammaproteobacterial methanotrophs seem to be more relevant (Cheema et al., 2015; Danilova & Dedysh, 2014; Dedysh, 2009).

2.4 Anaerobic CH_4 oxidation

Anaerobic CH_4 oxidation (AOM) was first discovered in marine sediments (Reeburgh, 1980) and later found to be performed by consortia of CH_4 oxidizing archaea (ANME) and SO_4^{2-}-reducing bacteria, or independently by archaea (Boetius et al., 2000). Three phylogenetic clusters of ANME have since been identified: ANME 1, ANME-2, and ANME-3 (Cui et al., 2015; Timmers et al., 2017). AOM coupled to SO_4^{2-} reduction with Deltaproteobacterial partners occurs in all three ANME groups (Cui et al., 2015; Timmers et al., 2017). Recently, it was demonstrated that NO_3^- (Haroon et al., 2013; Raghoebarsing et al., 2006), nitrite (Ettwig et al., 2010; Wu et al., 2011), iron (Fe^{3+}), and manganese (Mn^{4+}), are also potential electron acceptors in AOM (Ettwig et al., 2016). AOM coupled to NO_3^-, Fe^{3+}, and Mn^{4+} reduction occurs in the subgroup ANME-2d (Haroon et al., 2013). Nitrite-dependent AOM is performed by a proposed member of the NC 10 bacterial phylum, *Candidatus* 'Methylomirabilis oxyfera' (*Ca.* 'M. Oxyfera') that is found to co-occur with ANME-2d (Cui et al., 2015; Haroon et al., 2013; Timmers et al., 2017; Wu et al., 2011). This recently discovered organism produces its own oxygen via disproportionation of two NO molecules to N_2 and O_2. CH_4 is then oxidized using MMO and the classical pathway for aerobic CH_4 oxidation (Ettwig et al., 2010; Wu et al., 2011). Archaeal ANME are believed to perform CH_4 oxidation via reverse methanogenesis, though other pathways have been proposed as well (Caldwell et al., 2008; Hallam, 2004).

Until recently, SO_4^{2-}-dependent AOM was assumed to be irrelevant in terrestrial freshwater peatlands, due to low dissolved SO_4^{2-} concentrations (compared to the marine environment) and the presence of other, more energetically favorable electron acceptors (Caldwell et al., 2008; Timmers et al., 2016). Segarra et al. (2015) recently demonstrated the potential for high rates of SO_4^{2-}-dependent AOM in terrestrial peatlands. However, the importance of AOM in terrestrial peatlands has rarely been investigated (Zhu et al., 2012). AOM coupled to NO_3^- and/or nitrite reduction may be a more important process in rewetted temperate fens, because of a history of agricultural use (and therefore nitrogen input).

3 Methods and current understanding

3.1 Microbial community profiling

Historically, researchers relied primarily on culture studies to identify and enumerate microbes. In a microbial culture, an organism or a group of organisms is grown on a medium under controlled laboratory conditions. This remains an essential method in microbiology, but is limited because the vast majority of microbes cannot be isolated and/or cultured (Amann et al., 1995; Tang, 2020). To identify microbes in the environment, a number of "fingerprinting" techniques have thus been developed. Denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), temperature gradient gel electrophoresis (TGGE), automated ribosomal intergenic spacer analysis (ARISA), single-strand conformation polymorphism (SSCP), and phospholipid fatty acid (PLFA) are some examples of techniques that are still employed (Kirk et al., 2004; Rincon-Florez et al., 2013; van Dorst et al., 2014). Comparisons of these methods have shown they enable reliable characterization of microbial groups and identification of ecological patterns (van Dorst et al., 2014). Most of them, however, do not provide the opportunity to obtain direct taxonomic information (van Dorst et al., 2014), and are limited in their ability to detect less abundant taxa (Bent et al., 2007). Additionally, the wide variety of employed methods probably hinders the ability to make robust comparisons of trends among studies (Dunbar et al., 2001).

DNA sequencing – the process of determining the order of nucleotides in DNA – provides a basis for phylogenetic characterization of microorganisms into operational taxonomic units (OTUs), and thus provides direct taxonomic information. Traditional sequencing methods (e.g., Sanger Sequencing) were time-consuming and prohibitively expensive (Williams et al., 2015). Subsequent improvements and the development of high-throughput next generation sequencing (NGS) technologies have enabled the generation of massive taxonomic datasets for microbes in various environments, and have led to the discovery that previous methods underestimated microbial diversity by orders of magnitude (Sogin et al., 2006). NGS has since become standard practice for identification of microbial communities in the environment, though in certain cases, other fingerprinting methods maybe preferred, depending on the scope of the study (McDonald et al, 2008). Limitations of NGS include amplication bias introduced during PCR (Pinto & Raskin, 2012), formation of chimeras (Haas et al., 2011), and sequencing errors (Quince et al., 2011), the effects of which can be curtailed with careful experimental design and implementation (van Dorst et al., 2014). Additionally, it is believed sequencing may also underestimate microbial diversity because detection is dependent on the abundance of microbes (Tang, 2020).

The 16S rRNA gene is often targeted in sequencing studies of microbes in the environment because it is highly conserved in prokayrotes (bacteria and archaea; Amann, 1995; Coenye & Vandamme, 2003; Dziewit et al., 2015). Thus, whole microbial community profiles can be determined, and relative abundances estimated. In 16S rRNA sequencing, variable regions of an otherwise highly conserved gene are targeted and amplified using specific primers. For taxonomic classification, resulting sequences are compared to an existing database. The 16S rRNA gene is especially useful for classifying the overall microbial community, and identifying trends within it. For more in-depth diversity analyses, primers targeting a number of other specific marker genes such as *mcrA*, *pmoA*, among others, have been developed (Dziewit et al., 2015; McDonald et al., 2008).

There exist numerous bioinformatic methods and software packages for the processing of raw sequencing data. Most recently, assigned sequence variant (ASV) methods have eclipsed traditional OTU clustering methods. Using ASV methods, resulting sequences can be resolved down to a single nucleotide (Callahan et al., 2016).

3.2 Abundance analyses

Current techniques for enumerating specific groups of microbes include fluorescence in situ hybridization (FISH), analysis of intact polar lipids (IPLs), and quantitative polymerase chain reaction (qPCR, also called real-time PCR; Ramette, 2009; Wegener et al., 2016). qPCR is the current method of choice due to its sensitivity, reproducibility, and cost-effectiveness (Pabinger et al., 2014; Postollec et al., 2011). In qPCR, a target gene is tagged with a fluorescent marker and amplified. The change in fluorescence as the reaction proceeds provides a basis for the quantification of the target gene. The initial gene abundance is then calculated by the use of standard curves. The calculated gene abundances are used a proxy for the total abundance of the targeted organism (Higuchi et al., 1993).

3.3 Measurements of microbial activity

Microbial activity can be measured both directly and indirectly by a number of different methods. Respiration (i.e., CO_2 production) measurements are often used for estimation of total microbial activity, but can be cumbersome and expensive (Gyawali et al., 2019; Solaiman, 2007). Hydrolysis of fluorescein diacetate (FDA) was developed because it was simple to achieve, rapid, and sensitive (Schnürer & Rosswall, 1982), and earlier research found the majority of microbes perform FDA hydrolysis. Additionally, the technique is suited for a wide range of soils, making it an ideal non-specific estimate for overall soil microbial activity (Solaiman 2007). In this method, the breakdown of the FDA molecule by microbial enzymes is exploited. Fluorescein, the product of FDA hydrolysis, is quantified using spectrophotometry. Rates of FDA hydrolysis (and therefore microbial activity) can then be calculated (Schnürer and Rosswall 1982). To estimate activity of specific microbial groups, formation rates of expected end products can be measured. For example, methanogen activity can be estimated by determining rates of CH_4 formation (e.g, Sousa et al., 2013).

Advances in sequencing technologies have also improved assessment of active microbial populations. Microbial RNA can be extracted from an environmental sample, reverse-transcribed, and the resulting genetic material, called complementary DNA (cDNA), can be sequenced using the same technologies as for genomic DNA sequencing. Performed on total RNA, the method permits construction of community profiles depicting the active microbial community (Urich et al., 2008; Wemheuer et al., 2012). For a more direct assessment of gene expression, messenger RNA can be isolated from the total RNA pool, reverse transcribed, and sequenced (Freitag & Prosser, 2009).

3.4 Peatland microbial community dynamics in relation to water level

3.4.1 *Methanogen community dynamics*

The novelty of efficient methods for characterizing microbial groups in the environment dictates that our knowledge of *in situ* peatland microbial community dynamics is still limited. Earlier culture and incubations studies have shown that oxygen introduction can cause methanogen mortality, and/or decrease rates of CH_4 production (Dowrick et al., 2006; Kiener & Leisinger, 1983; Knorr & Blodau, 2009). Changing water levels thus affect methanogens by regulating oxygen penetration into the peat, potentially inducing metabolic stress related to oxygen toxicity. Additionally, oxygen introduction can replenish TEAs, which may then be used by organisms that are able to outcompete methanogens for substrate (Roden & Wetzel, 2002; Segers, 1998). Evidence for this exists in incubation studies have shown that, following drought treatments, TEAs were renewed, while methanogenesis was suppressed even for some time following rewetting (Dowrick et al., 2006; Knorr and Blodau; 2009). More recently, *in* *situ* studies have shown water level changes can induce shifts in the community composition of archaea (Tian et al., 2012 and 2015) and of methanogens specifically (Urbanová & Bárta, 2020), as well as alter total and relative methanogen abundances (Urbanová & Bárta, 2020; Wang et al., 2020; Weil et al., 2020).

Significantly higher methanogen abundances linked to water level increases have been measured in bogs (Reumer et al., 2018), rice paddy soils (Reim et al., 2017), and fens (Weil et al., 2020; Wen and Unger et al., 2018) while lower methanogen abundances have been linked to water level reduction (Ma & Lu, 2011; Tian et al., 2012, 2015). Evidence increasingly suggests, however, microbial community dynamics – and environmental alterations to them – may be site specific. Interestingly, in a study of boreal fens, methanogen abundances were found to be lower in restored (i.e., rewetted) sites compared to drained sites (Juottonen et al., 2012). Kim et al. (2008) found that drought treatments reduced methanogen abundances in bog, but not in fen or riparian peat mesocosms. Additionally, Peltoniemi et al. (2016) found that warming and drying treatments decreased methanogen abundances in some, but not all areas of two boreal fens.

3.4.2 *Methanotroph community dynamics*

The effect of water level changes on methanotroph community dynamics is also not well-resolved. Aerobic methanotrophs thrive on the oxic/anoxic interface where oxygen and CH_4 are both readily available (Knief, 2015), and water level changes can therefore alter substrate and oxygen availability. Studies focusing on methanotroph community dynamics under changing hydrological conditions have increased in number in recent years (Krause et al., 2015; Peltoniemi et al., 2016), but the vast majority of studies have been experimental, conducted as either incubations (Collet et al., 2015; Ho et al., 2016;a Ho et al., 2016b) mesocosms (Kim et al., 2008), or, rarely, field manipulations (Peltoniemi et al., 2016). Early research showed that rates of CH_4 oxidation are higher when water level is below the peat surface, and increase following water level decrease (Roslev & King, 1996). Incubations have also demonstrated that temporary water level decreases can promote CH_4 oxidation (Knorr et al., 2008). This is supported by other studies which suggest some aerobic methanotrophs benefit from water level reduction and modest soil drying (Henckel et al., 2001; Ma & Lu, 2011; Peltoniemi et al., 2016, Unger et al., *submitted*). Aerobic methanotrophs have demonstrated a remarkable ability to recover from environmental stress related to water level changes (Bodelier et al., 2011; Collet et al., 2015; Henckel et al., 2001; Ho et al., 2013a, b; Ho et al., 2016a, b; Pan et al., 2014; Roslev

& King, 1996) However, methanotroph community dynamics have rarely been investigated *in situ* in this context (Wen and Unger et al., 2018, Unger et al., *submitted*).

Similar to methanogens, recent studies highlight the complexity of microbial respone to water level changes. For example, Peltoniemi et al. (2016) detected changes in aerobic methanotroph community composition in one of two boreal fens subject to warming and drying treatments. Krause et al. (2015) found methanotroph community composition to be similar among drained and rewetted acidic Dutch peatlands, while short term-flooding did not change the abundances of the detected aerobic methanotrophs. Furthermore, an increasing number of studies suggest a differential response to water level changes among methanotroph types, revealing more complex community dynamics than previously anticipated. A handful of incubation experiments have demonstrated type I methanotrophs may be more responsive to changing hydrological conditions than type II methanotrophs (Bodelier et al., 2011; Collet et al., 2015; Henkel et al., 2001; Ho et al., 2013a, b; Ho et al., 2016a, b; Pan et al., 2014). Supporting this observation, in their field manipulation experiment Peltoniemi et al. (2016) found a combination of warming and drying decreased the abundance of some type I methanotrophs, while differences in the abundance of type II methanotrophs could only be explained by depth, and not by warming and/or drying treatments.

4 Aims of this thesis

To date, most studies of microbial methane cycling in rewetted peatlands have been conducted in bogs and nutrient-poor fens. Furthermore, most studies examining water level effects on CH_4-cycling microbes have been conducted in a lab setting. Therefore, the overall goal of this body of work is to elucidate *in situ* patterns in microbial community dynamics (namely, community composition, abundances, and activity) in rewetted temperate fens, in the framework of CH_4 cycling. The specific goals were:

I. To characterize peat microbial community composition in rewetted fens, and determine if patterns in methanogen and methanotroph community composition and abundances reflect observed ecosystem CH_4 emissions

II. To determine if long-term drainage leads to decreased polyphenol content, higher microbial activity, and potentially higher CH_4 production in rewetted fen peats

III. To characterize the community dynamics of CH_4-cycling microbes during a natural drought

To this end, I conducted four studies together with colleagues. First, we employed NGS and qPCR, as well as geochemical analyses to compare microbial community composition and abundances in two rewetted, minerotrophic temperate fens (a coastal brackish and a freshwater riparian fen) with high CH_4 emissions (Appendix A), with a focus on methanogens and methanotrophs. Next, we utilized NGS and geochemical analyses to further investigate the conditions enabling high CH_4 emissions in the coastal fen, despite locally high SO_4^{2-} concentrations in the peat pore water (Appendix B), focusing on methanogens and SO_4^{2-} reducers. Then, we compared polyphenol content in peats from natural (i.e., not or hardly disturbed) fens and rewetted fens to determine if long-term drainage led to a reduction in microbe-inhibiting polyphenols (Appendix C). We further investigated the relationship between polyphenol content and microbial activity using FDA hydrolysis and enzyme inhibition tests. Finally, during the 2018 European summer drought, we employed NGS of DNA and cDNA reverse transcribed from total RNA, as well as qPCR, to characterize the *in situ* microbial community dynamics underlying reduced CH_4 emissions in fens during a natural drought event (Appendix D). We conducted this study in the same two fens investigated in Appendix A. Therefore, we were able to compare microbial community dynamics among a drought and non-drought year and assess potential changes in the *in situ* CH_4-cycling community.

5 Synthesis

5.1 Results and implications

5.1.1 *Predominance of methanogens over methanotrophs in rewetted fens*

In Wen and Unger et al. (2018, Appendix A), we found a high abundance of methanogens and high methanogen/methanotroph ratios to be characteristic of both a coastal brackish (Hütelmoor) and a freshwater riparian fen (Zarnekow), despite differing environmental conditions and overall microbial community composition. Porewater geochemical analyses showed the Hütelmoor was characterized by higher electrical conductivity and TEA availability, especially oxygen in the upper peat layer. The differing environmental conditions of the two fens were also apparent in the sequencing data, as NMDS analysis showed that bacterial, archaeal, and total microbial community composition differed among the two sites, and was influenced by the measured environmental parameters.

Nevertheless, community dynamics of methanogens and methanotrophs were similar among the two fens. 16S rRNA gene sequencing and qPCR showed methanogens (mostly acetoclastic) were similarly (and highly) abundant in both fens. Methanotrophs, meanwhile, were underrepresented in the bacterial community profiles, representing only 0.05 and 0.06 % of total bacterial community in Zarnekow and Hütelmoor peat, respectively. Based on qPCR, we calculated a high ratio of methanogens to methanotrophs, especially in the surface peat where ratios were as high as ~1500. For comparison, Liebner et al. (2015), for example, found methanotrophs to represent 0.5 % of the total bacterial community in a pristine subarctic transitional bog-fen palsa, while *mcrA* and *pmoA* gene copy numbers were nearly identical. Furthermore, our results are in line with Juottonen et al. (2012), who measured a lower abundance of methanotrophs in rewetted sites compared to drained sites, in a study of boreal fens. The high methanogen:methanotroph ratios we found in this study may be a characteristic of rewetted temperate fens with high CH_4 emissions.

This was the first study to demonstrate the predominance of CH_4-producers over CH_4-consumers in rewetted temperate fens with high CH_4 emissions. Previous studies documented a drastic increase in CH_4 fluxes in rewetted temperate fens (Hahn et al., 2015), which was partially attributed to a large increase in easily degradable dissolved organic matter following rewetting (Hahn-Schöfl et al., 2011). The dominance of acetoclastic methanogens at the two sites in this study supports this implication. However, this study further shows that CH_4-consumers were underrepresented in the bacterial community profiles, suggesting post-rewetting conditions potentially hindered their growth. Possible reasons for this could be high ammonium concentrations, which have been measured in rewetted fen peat (Emsens et al., 2016; Lundin et al., 2017; Zak & Gelbrecht, 2007), and/or competition with other microbes (van Bodegom et al., 2001). In rice paddy soils, van Bodegom et al. (2001) have shown that at high acetate concentrations, methanotrophs may be outcompeted by heterotrophs for substrate. In Wen and Unger at al., (2018, Appendix A), bacterial community profiles revealed a dominance of heterotrophic bacteria.

The results of Wen and Unger et al. (2018) show that studies of CH_4-cycling microbes can provide additional insight into observed high CH_4 fluxes in rewetted fens. The ultimate goal of rewetting peatlands is a return to natural or near-natural conditions, and reestablish the C sink function. In this context, GHG emissions, nutrient cycling (e.g., phosphorus and nitrogen) and plant community dynamics are typically monitored, while microbial community dynamics are often not considered. Information gained from monitoring microbial community dynamics during rewetting projects would help refine predictions to changes in the biogeochemical cycling of CH_4, and other GHGs, before rewetting projects are implemented. Based on these results, there seems to be a need to more directly assess the effects rewetting has on methanotrophs If possible, future studies should attempt to analyze peat microbial communities before and after rewetting, with a special focus on methanotrophs. Additionally, incubation studies that attempt to disentangle the potential influence of ammonium, acetate, and/or competition with heterotrophs could be conducted. Ideally, a combination of community profiling and techniques for quantification of specific microbial groups should be utilized, along with relevant geochemical parameters and rates of CH_4 production, oxidation, and/or emission.

5.1.2 *Sulfate deprivation triggers high methane production in a rewetted coastal fen*

In Koebsch et al. (2019, Appendix B), we found SO_4^{2-}-induced inhibition of CH_4 production to be an insignificant process in the previously studied, rewetted coastal fen. Across all measurement spots along a land-sea transect, dissolved SO_4^{2-} concentrations did not exceed 0.3 mM in the top 20 cm of peat, and, at the same depths, SO_4^{2-} reducing bacteria were completely absent or low in abundance (< 4 % of bacterial community). Acetoclastic methanogens of *Methanosaeta*, a genus common in freshwater peatlands, dominated the upper peat layers, representing up to ~75 % of the archaeal community. Former brackish impact was evident from high sedimentary S concentrations and the ^{34}S composition of the remaining SO_4^{2-}. However, rewetting of the coastal fen with freshwater apparently promoted biogeochemical conditions in the upper peat layer similar to that of terrestrial freshwater wetlands. Like Wen and Unger et al. (2018, Appendix A), this study shows how microbial community dynamics can provide a deeper insight into mechanisms that control CH_4 exchange in rewetted fens.

Coastal wetlands are typically assumed to be CH_4 sinks because of SO_4^{2-} input of marine origin. The results of this study suggest that inundation of coastal brackish peatlands with freshwater has the potential to induce SO_4^{2-} limited conditions and promote methanogenesis. Coastal peatlands are highly impacted by human influence, but interest in preserving these systems to maintain biodiversity and protect coastlines from erosion is gaining traction. The results from this study have implications for management decisions regarding the source of water used to rewet coastal peatlands. Rewetting with sea water rather than fresh water may promote the growth of SO_4^{2-}-reducing organisms, which could potentially outcompete methanogens and reduce post-rewetting CH_4 emissions. Given these results, CH_4 emissions from coastal systems may be underestimated if human activity causes natural CH_4 inhibition mechanisms to cease.

5.1.3 *Lower polyphenol content and higher microbial activity in rewetted fen peats*

In Zak et al., (2019, Appendix C), we found that total polyphenols and condensed tannins (i.e., high molecular weight polyphenols with multiple phenolic hydroxyl groups) were eight and fifty times lower in highly degraded, rewetted fen peats, respectively. We conducted enzyme inhibition tests using extracted polyphenols on fen peats from Zarnekow, and found no clear relationship between polyphenol content and microbial activity. However, the samples with the lowest polyphenol content had the highest levels of microbial activity, as determined with FDA hydrolysis. The results suggest that a reduction in polyphenol content from long-term drainage could contribute to higher microbial activity – and therefore CH_4 turnover – in rewetted fens. Thus, the enzymatic “latch” hypothesis can also be applied to fens.

The non-specific nature of FDA hydrolysis probably contributed to our inability to draw a clear relationship between microbial activity and polyphenol content. Additionally, different types of polyphenols may have a greater (or lesser) inhibitory effect on microbes due to their differing chemical structures. However, as the role of polyphenols in fens has rarely been investigated, the broad scope of this study provides a necessary first look into the dynamics of polyphenol accumulation and microbial activity in fen peats. Though we did not analyze the effect of polyphenols on methanogen activity in this study, previous studies have shown polyphenols can inhibit methanogen activity. To determine if polyphenol degradation promotes higher CH_4 emissions in rewetted peatlands, a similar study could be conducted, measuring instead CH_4 production rates in peats with various concentrations of polyphenols, rather than total enzymatic activity. This would be an important next step in understanding the increase in CH_4 emissions that usually follows rewetting in fen peatlands. Topsoil removal (i.e., removal of the highly degraded peat layer) before rewetting could potentially mitigate the effects of polyphenol degradation and reduce nutrient and GHG turnover in rewetted peats. Topsoil removal has been shown to be effective in reducing CH_4 emissions in rewetted bogs (Huth et al., 2020) and reducing ammonium (and other nutrient) concentrations in minerotrophic fens (Emsens et al., 2015).

5.1.4 *Community dynamics of methanogens and methanotrophs reflect decreased ecosystem CH_4 emission during a natural drought.*

In Unger et al., (*submitted,* Appendix D), the drought-related decrease in CH_4 emissions was reflected in the microbial community as differences in the total and relative abundances of methanogens and methanotrophs. Along with a dramatic decrease in average water level and ecosystem scale CH_4 emissions in both the coastal and riparian fen, we measured a higher relative abundance of type I methanotrophs in both fens during drought (compared to a non-drought year). 16S rRNA gene sequencing showed type I aerobic methanotrophs of the order *Methylococcales* represented up to 6 %, and averaged 1.1 % of the total bacterial community. Type II methanotrophs (mainly *Methylocystis*) relative abundances were low (≤ 0.1 %), and similar to the non-drought year. Community profiles developed from reverse transcription of total RNA and subsequent cDNA sequencing showed that type I aerobic methanotrophs were the dominant active bacterial group in both fens during drought, with an average relative abundance of 8 %. Differences in the total abundance of methanogens and methanotrophs, and overall microbial community composition, however, were site specific. Total methanogen abundance based on *mcrA* gene copy numbers was significantly lower in Zarnekow during drought, while total methanotroph abundance based on *pmoA* gene copy numbers was higher in the Hütelmoor. Though overall community dynamics differed among the fens, type I methanotrophs apparently recovered in both fens, despite being nearly absent after rewetting. Thus, type I methanotrophs may represent an important microbial control over CH_4 emissions in fens subject to natural drought.

The dominance of type I methanotrophs during drought, compared to their near absence under inundated conditions, suggests the drought may have in some way stimulated their growth and/or activity. More studies are needed to confirm this. If type I methanotrophs are indeed stimulated by short-term drops in water level, this could have implications for management decisions regarding rewetting projects. Often, drained peatlands are immediately, and fully flooded, resulting in continuously inundated conditions in many cases. Pulse-flooding (i.e., intermittent flooding) has been investigated as a potential method to reduce the peak in CH_4 emissions measured during the rewetting process (Hu et al., 2017). In a study of created wetlands, Altor & Mitsch (2008) found CH_4 emissions were 2 times lower under pulse-flooding compared to steady-flow conditions. In Wen and Unger et al., (2018, Appendix A), we already show how continuously inundated conditions may negatively affect methanotroph establishment. Pulse-rewetting might provide better chance for methanotrophs, specifically type I methanotrophs, to establish themselves during the rewetting process.

This was the first paper to characterize *in situ* methanogen and methanotroph community dynamics in rewetted temperate fens subject to a natural (i.e., non-experimental) drought. It suggests that reduced CH_4 emissions in restored fens subject to natural drought are at least partially the result of the different controlling patterns of methanogens and methanotrophs, which has been noted in previous drought studies (e.g., Peltoniemi et al., 2016). Previous, however, were mostly experimental, and conducted *ex situ* as incubation or mesocosm studies. Several of these studies suggested that type I methanotrophs are more responsive to drought effects (i.e., drying and temperature increase), and are able to rapidly increase in population size in suitable conditions. This study provides the first *in situ* evidence (from a natural drought) supporting these observations, and adds to a growing body of evidence which suggests differing life strategies among methanotroph types. This has implications for future studies of methanotrophs and CH_4 oxidation in peatlands, because it highlights the importance of considering specific microbial taxa in studies of peatland GHG exchange under changing water levels. Ideally, once the important taxa are identified, follow-up studies could be conducted in the lab, in which the microbial groups are subject to controlled manipulations.

5.2 Conclusions and outlook

Microbial community dynamics have rarely been investigated rewetted temperate fens, ecosystems which tend to be a significant CH_4 source for some time following restoration. With this thesis, I attempted to address this by using state-of-the-art microbial community profiling and enumeration techniques, as well as supplemental activity investigations, to elucidate patterns in microbial community dynamics in these systems. In Wen and Unger et al. (2018, Appendix A), we show that low methanotroph abundances, and high methanogen/methanotroph ratios may be a characteristic of rewetted temperate fens with high CH_4 emissions, while in Zak et al. (2019, Appendix C), we show that higher microbial activity (and probably CH_4 turnover) can be expected in rewetted fens. In Koebsch et al. (2019), we further reveal a lack of available SO_4^{2-} (probably induced by rewetting with freshwater) and low abundances of SRB to be underlying characteristics of the rewetted coastal fen discussed in Wen and Unger et al. (2018) and Unger et al. (*submitted,* Appendix D). In the latter, we demonstrate that a drought-related decrease in ecosystem CH_4 emissions corresponded with a large increase in the relative abundance of methanotrophs. Similar to the underrepresentation of methanotrophs discovered in Wen and Unger et al. (2018, Appendix A), this finding was true for both the coastal fen and riparian fen that were investigated, despite inherently different environmental settings and porewater geochemistry. Overall, these findings represent an important first step in understanding the microbial groups influencing CH_4 cycling in rewetted temperate fens.

More studies are needed to confirm whether or not these findings are specific to the studied systems, or can be expected in other temperate fens. The similar findings in two different fen types in Wen and Unger et al., (2018) and Unger et al. (*submitted*), and five rewetted fens in Zak et al. (2019), strengthens the postulation that some of the observed differences in microbial community composition, abundances, and/or activity could be expected among temperate fens in general. Some studies suggest that and environmental changes to microbial community dynamics may be site specific (Kim et al., 2008, Peltoniemi et al., 2016), however. This thesis also contains evidence supporting this observation. For example, in Unger et al. (*submitted*, Appendix D) we expected to detect a lower total abundance of methanogens during drought in both fens, as had been found in previous studies. We only found this to be true for one of the studied fens, however. Importantly, these findings do not necessarily dictate that similar trends do not exist among peatlands. Rather, they emphasize the need for a larger number of studies from which to compare findings. The findings in this thesis provide a basis for future studies which should attempt to relate microbial community dynamics to site-specific conditions.

The analyses performed in this thesis provide fundamental insights on the *in situ* microbial community dynamics of a widespread, but understudied peatland type, and highlight the value of microbially-focused studies in interpreting, and potentially anticipating changes in fen CH_4 cycling under changing water levels. On their own, however, such analyses do not directly link changes in microbial community dynamics to changes in CH_4 flux rates, and fall short of providing a direct, quantitative link to CH_4 transformation. Using 16S rRNA gene sequencing on environmental samples (i.e., peat), the resident microbial community can be classified and their relative abundances estimated. Without additional information, we can only speculate about which microbial groups are performing the majority of, for example, CH_4 production or consumption. A promising technique currently used for this purpose is stable isotope probing (SIP). In addition to enabling isolation and identification of active microbes, SIP can be use to determine uptake rates of labeled substrate, providing some quantitative information. Furthermore, the technique can be performed *in situ* (Lu & Conrad, 2005), eliminating the inherent bias of controlled incubation experiments.

Ultimately, a comprehensive understanding of microbial groups and their specific controls over CH_4 production, oxidation, and emissions can only be achieved with a combination of methods that examine microbial community dynamics and processes at different scales. As the studies accumulate and the gaps in knowledge are filled, models can be further constrained, and predictions to water level-induced alterations to GHG dynamics can be refined.

5.3 Final remarks

Here, I shed light on patterns in microbial community composition, abundances, and activity in rewetted temperate fens, in an attempt to better understand observed patterns in CH_4 cycling. To date, the majority of similar studies have focused on bogs and nutrient-poor fens. With regards to CH_4 cycling and microbial community dynamics in temperate fens, we are still in the early phases of research. However, this can also be attributed to a historical lack of appropriate methodologies for analysis of microbial community in the environment. The state-of-the-art analyses utilized in this thesis, particularly NGS and qPCR, are constantly under improvement, and, in combination with other techniques, have the potential to reveal vast amounts of information on microbial communities and GHG exchange in the environment. Such information will be essential in refining predictions to changes in the cycling of CH_4 and other GHGs as researchers attempt to assess the compounding effects of land-use changes and climate change effects in the near future. Data from such studies could be used to develop more robust models which attempt to upscale microbial processes beyond the landscape level.

6 References

Abdalla, M., Hastings, A., Truu, J., Espenberg, M., Mander, Ü., & Smith, P. (2016). Emissions of methane from northern peatlands: a review of management impacts and implications for future management options. *Ecology and Evolution*, *6*(19), 7080–7102. https://doi.org/10.1002/ece3.2469

Altor, A. E., & Mitsch, W. J. (2008). Pulsing hydrology, methane emissions and carbon dioxide fluxes in created marshes: A 2-year ecosystem study. *Wetlands*, *28*(2), 423–438. https://doi.org/10.1672/07-98.1

Amann, R. I., Ludwig, W., & Schleifer, K.-H. (1995). Phylogenetic Identification and In Situ Detection of Individual Microbial Cells without Cultivation. *Microbiological Reviews*, *59*(1), 143–169.

Amann, Rudolf I. (1995). In situ identification of micro-organisms by whole cell hybridization with rRNA-targeted nucleic acid probes. In A. D. L. Akkermans, J. D. Van Elsas, & F. J. De Bruijn (Eds.), *Molecular Microbial Ecology Manual* (pp. 331–345). Springer. https://doi.org/10.1007/978-94-011-0351-0_23

Augustin, J., Merbach, W., & Rogasik, J. (1998). Factors influencing nitrous oxide and methane emissions from minerotrophic fens in northeast Germany. *Biology and Fertility of Soils*, *28*, 1–4. https://doi.org/10.1007/s003740050455

Baird, A. J., Beckwith, C. W., Waldron, S., & Waddington, J. M. (2004). Ebullition of methane-containing gas bubbles from near-surface Sphagnum peat. *Geophysical Research Letters*, *31*(21). https://doi.org/10.1029/2004GL021157

Bent, S. J., Pierson, J. D., Forney, L. J., Danovaro, R., Luna, G. M., Dell'Anno, A., & Pietrangeli, B. (2007). Measuring Species Richness Based on Microbial Community Fingerprints: the Emperor Has No Clothes. *Applied and Environmental Microbiology*, *73*(7), 2399–2401. https://doi.org/10.1128/AEM.02383-06

Bižić, M., Klintzsch, T., Ionescu, D., Hindiyeh, M. Y., Günthel, M., Muro-Pastor, A. M., Eckert, W., Urich, T., Keppler, F., & Grossart, H. P. (2020). Aquatic and terrestrial cyanobacteria produce methane. *Science Advances*, *6*(3), 1–9. https://doi.org/10.1126/sciadv.aax5343

Bodelier, P. L. E., Bär-Gilissen, M.-J., Meima-Franke, M., & Hordijk, K. (2011). Structural and functional response of methane-consuming microbial communities to different flooding regimes in riparian soils. *Ecology and Evolution*, *2*(1), 106–127. https://doi.org/10.1002/ece3.34

Boetius, A., Ravenschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B. B., Witte, U., & Pfannkuche, O. (2000). A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, *407*(6804), 623–626. https://doi.org/10.1038/35036572

Borrel, G., O'Toole, P. W., Harris, H. M. B., Peyret, P., Brugère, J. F., & Gribaldo, S. (2013). Phylogenomic data support a seventh order of methylotrophic methanogens and provide insights into the evolution of methanogenesis. *Genome Biology and Evolution*, *5*(10), 1769–1780. https://doi.org/10.1093/gbe/evt128

Bowman, J. (2006). The Methanotrophs — The Families Methylococcaceae and Methylocystaceae. In *The Prokaryotes* (pp. 266–289). Springer New York. https://doi.org/10.1007/0-387-30745-1_15

Cadillo-Quiroz, H., Yashiro, E., Yavitt, J. B., & Zinder, S. H. (2008). Characterization of the archaeal community in a minerotrophic fen and terminal restriction fragment length polymorphism-directed isolation of a novel hydrogenotrophic methanogen. *Applied and Environmental Microbiology*, *74*(7), 2059–2068. https://doi.org/10.1128/AEM.02222-07

Caldwell, S. L., Laidler, J. R., Brewer, E. A., Eberly, J. O., Sandborgh, S. C., & Colwell, F. S. (2008). Anaerobic oxidation of methane: Mechanisms, bioenergetics, and the ecology of associated microorganisms. *Environmental Science and Technology*, *42*(18), 6791–6799. https://doi.org/10.1021/es800120b

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Cheema, S., Zeyer, J., & Henneberger, R. (2015). Methanotrophic and methanogenic communities in swiss alpine fens dominated by Carex rostrata and Eriophorum angustifolium. *Applied and Environmental Microbiology*, *81*(17), 5832–5844. https://doi.org/10.1128/AEM.01519-15

Clymo, R. S. (1984). The limits to peat bog growth. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *303*, 605– 654.

https://doi.org/10.1098/rstb.1984.0002

Coenye, T., & Vandamme, P. (2003). Intragenomic heterogeneity between multiple 16S ribosomal RNA operons in sequenced bacterial genomes. *FEMS Microbiology Letters*, *228*, 45–49. https://doi.org/10.1016/S0378-1097(03)00717-1

Collet, S., Reim, A., Ho, A., & Frenzel, P. (2015). Recovery of paddy soil methanotrophs from long term drought. *Soil Biology and Biochemistry*, *88*, 69–72. https://doi.org/10.1016/j.soilbio.2015.04.016

Conrad, R. (2007). Microbial Ecology of Methanogens and Methanotrophs. *Advances in Agronomy*, *96*, 1–63. https://doi.org/10.1016/S0065-2113(07)96005-8

Cui, M., Ma, A., Qi, H., Zhuang, X., & Zhuang, G. (2015). Anaerobic oxidation of methane: an “active” microbial process. *MicrobiologyOpen*, *4*(1), 1–11. https://doi.org/10.1002/mbo3.232

Danilova, O. V., & Dedysh, S. N. (2014). Abundance and diversity of methanotrophic Gammaproteobacteria in northern wetlands. *Microbiology*, *83*(1–2), 67–76. https://doi.org/10.1134/S0026261714020040

Dean, J. F., Middelburg, J. J., Röckmann, T., Aerts, R., Blauw, L. G., Egger, M., Jetten, M. S. M., de Jong, A. E. E., Meisel, O. H., Rasigraf, O., Slomp, C. P., in’t Zandt, M. H., & Dolman, A. J. (2018). Methane Feedbacks to the Global Climate System in a Warmer World. *Reviews of Geophysics*, *56*(1), 207–250. https://doi.org/10.1002/2017RG000559

Dedysh, S. N. (2009). Exploring methanotroph diversity in acidic northern wetlands: Molecular and cultivation-based studies. *Microbiology*, *78*(6), 655–669. https://doi.org/10.1134/S0026261709060010

Dedysh, Svetlana N., & Dunfield, P. F. (2016). Beijerinckiaceae. In *Bergey’s Manual of Systematics of Archaea and Bacteria*. John Wiley & Sons, Inc. https://doi.org/10.1002/9781118960608.fbm00164.pub2

Dowrick, D. J., Freeman, C., Lock, M. A., & Reynolds, B. (2006). Sulphate reduction and the suppression of peatland methane emissions following summer drought. *Geoderma*, *132*(3–4), 384–390. https://doi.org/10.1016/j.geoderma.2005.06.003

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*(1), 190–197. https://doi.org/10.1128/AEM.67.1.190-197.2001

Dziewit, L., Pyzik, A., Romaniuk, K., Sobczak, A., Szczesny, P., Lipinski, L., Bartosik, D., & Drewniak, L. (2015). Novel molecular markers for the detection of methanogens and phylogenetic analyses of methanogenic communities. *Frontiers in Microbiology*, *6*. https://doi.org/10.3389/fmicb.2015.00694

Emsens, W.-J., Aggenbach, C. J. S., Schoutens, K., Smolders, A. J. P., Zak, D., & Van Diggelen, R. (2016). Soil iron content as a predictor of carbon and nutrient mobilization in rewetted fens. *PLoS ONE*, *11*, 1–17. https://doi.org/10.1371/journal.pone.0153166

Emsens, W.-J., van Diggelen, R., Aggenbach, C. J. S., Cajthaml, T., Frouz, J., Klimkowska, A., Kotowski, W., Kozub, L., Liczner, Y., Seeber, E., Silvennoinen, H., Tanneberger, F., Vicena, J., Wilk, M., & Verbruggen, E. (2020). Recovery of fen peatland microbiomes and predicted functional profiles after rewetting. *ISME Journal*, *14*, 1701–1712. https://doi.org/10.1038/s41396-020-0639-x

Emsens, W. J., Aggenbach, C. J. S., Smolders, A. J. P., & van Diggelen, R. (2015). Topsoil removal in degraded rich fens: Can we force an ecosystem reset? *Ecological Engineering*, *77*, 225–232. https://doi.org/10.1016/j.ecoleng.2015.01.029

Ettwig, K. F., Butler, M. K., Le Paslier, D., Pelletier, E., Mangenot, S., Kuypers, M. M. M., Schreiber, F., Dutilh, B. E., Zedelius, J., de Beer, D., Gloerich, J., Wessels, H. J. C. T., van Alen, T., Luesken, F., Wu, M. L., van de Pas-Schoonen, K. T., Op den Camp, H. J. M., Janssen-Megens, E. M., Francoijs, K.-J., … Strous, M. (2010). Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature*, *464*(7288), 543–548. https://doi.org/10.1038/nature08883

Ettwig, K. F., Zhu, B., Speth, D., Keltjens, J. T., Jetten, M. S. M., & Kartal, B. (2016). Archaea catalyze iron-dependent anaerobic oxidation of methane. *Proceedings of the National Academy of Sciences*, *113*(45), 12792–12796. https://doi.org/10.1073/pnas.1609534113

Evans, P. N., Boyd, J. A., Leu, A. O., Woodcroft, B. J., Parks, D. H., Hugenholtz, P., & Tyson, G. W. (2019). An evolving view of methane metabolism in the Archaea. *Nature Reviews Microbiology*, *17*, 219–232. https://doi.org/10.1038/s41579-018-0136-7

Evans, P. N., Parks, D. H., Chadwick, G. L., Robbins, S. J., Orphan, V. J., Golding, S. D., & Tyson, G. W. (2015). Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics. *Science*, *350*(6259), 434–438. https://doi.org/10.1126/science.aac7745

Fenchel, T., Blackburn, T., & King, G. (2012). Bacterial biogeochemistry: the ecophysiology of mineral cycling. In *Bacterial biogeochemistry: The ecophysiology of mineral cycling* (3rd ed.). Academic Press. https://doi.org/10.1016/C2010-0-67238-5

Franchini, A. G., Henneberger, R., Aeppli, M., & Zeyer, J. (2015). Methane dynamics in an alpine fen: A field-based study on methanogenic and methanotrophic microbial communities. *FEMS Microbiology Ecology*, *91*(3), 1–13. https://doi.org/10.1093/femsec/fiu032

Freeman, C., Ostle, N., & Kang, H. (2001). An enzymic "latch" on a global carbon store: A shortage of oxygen locks up carbon in peatlands by restraining a single enzymes. *Nature*, *409*(6817), 149. https://doi.org/10.1038/35051650

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*(21), 6679–6687. https://doi.org/10.1128/AEM.01021-09

Galand, P. E., Fritze, H., Conrad, R., & Yrjälä, K. (2005). Pathways for Methanogenesis and Diversity of Methanogenic Archaea in Three Boreal Peatland Ecosystems. *Applied and Environmental Microbiology*, *71*(4), 2195–2198. https://doi.org/10.1128/AEM.71.4.2195

Günther, A., Barthelmes, A., Huth, V., Joosten, H., Jurasinski, G., Koebsch, F., & Couwenberg, J. (2020). Prompt rewetting of drained peatlands reduces climate warming despite methane emissions. *Nature Communications*, *11*, 1–5. https://doi.org/10.1038/s41467-020-15499-z

Gyawali, A. J., Lester, B. J., & Stewart, R. D. (2019). Talking SMAAC: A new tool to measure soil respiration and microbial activity. *Frontiers in Earth Science*, *7*. https://doi.org/10.3389/feart.2019.00138

Haas, B. J., Gevers, D., Earl, A. M., Feldgarden, M., Ward, D. V., Giannoukos, G., Ciulla, D., Tabbaa, D., Highlander, S. K., Sodergren, E., Methe, B., DeSantis, T. Z., Petrosino, J. F., Knight, R., & Birren, B. W. (2011). Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Research*, *21*(3), 494–504. https://doi.org/10.1101/gr.112730.110

Hahn-Schöfl, M., Zak, D., Minke, M., Gelbrecht, J., Augustin, J., & Freibauer, A. (2011). Organic sediment formed during inundation of a degraded fen grassland emits large fluxes of CH4 and CO2. *Biogeosciences*, *8*(6), 1539–1550. https://doi.org/10.5194/bg-8-1539-2011

Hahn, J., Köhler, S., Glatzel, S., & Jurasinski, G. (2015). Methane exchange in a coastal fen in the first year after flooding - A systems shift. *PLoS ONE*, *10*(10), 1–25. https://doi.org/10.1371/journal.pone.0140657

Hallam, S. J. (2004). Reverse Methanogenesis: Testing the Hypothesis with Environmental Genomics. *Science*, *305*(5689), 1457–1462. https://doi.org/10.1126/science.1100025

Hanson, R. S., & Hanson, T. E. (1996). Methanotrophic bacteria. *Microbiological Reviews*, *60*(2), 439–471. https://doi.org/10.1128/mmbr.60.2.439-471.1996

Haroon, M. F., Hu, S., Shi, Y., Imelfort, M., Keller, J., Hugenholtz, P., Yuan, Z., & Tyson, G. W. (2013). Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. *Nature*, *500*, 567–570. https://doi.org/10.1038/nature12375

Henckel, T., Jäckel, U., & Conrad, R. (2001). Vertical distribution of the methanotrophic community after drainage of rice field soil. *FEMS Microbiology Ecology*, *34*, 279–291. https://doi.org/10.1016/S0168-6496(00)00105-7

Ho, A., Kerckhof, F.-M., Luke, C., Reim, A., Krause, S., Boon, N., & Bodelier, P. L. E. (2013a). Conceptualizing functional traits and ecological characteristics of methane-oxidizing bacteria as life strategies. *Environmental Microbiology Reports*, *5*(3), 335–345. https://doi.org/10.1111/j.1758-2229.2012.00370.x

Ho, A., Lüke, C., Reim, A., & Frenzel, P. (2013b). Selective stimulation in a natural community of methane oxidizing bacteria: Effects of copper on pmoA transcription and activity. *Soil Biology and Biochemistry*, *65*, 211–216. https://doi.org/10.1016/j.soilbio.2013.05.027

Ho, A., Brink, E. van den, Reim, A., Krause, S. M. B., & Bodelier, P. L. E. (2016a). Recurrence and frequency of disturbance have cumulative effect on methanotrophic activity, abundance, and community structure. *Frontiers in Microbiology*, *6*, 1–10. https://doi.org/10.3389/fmicb.2015.01493

Ho, A., Lüke, C., Reim, A., & Frenzel, P. (2016b). Resilience of (seed bankb) aerobic methanotrophs and methanotrophic activity to desiccation and heat stress. *Soil Biology and Biochemistry*, *101*, 130–138. https://doi.org/10.1016/j.soilbio.2016.07.015

Huth, V., Günther, A., Bartel, A., Hofer, B., Jacobs, O., Jantz, N., Meister, M., Rosinski, E., Urich, T., Weil, M., Zak, D., & Jurasinski, G. (2020). Topsoil removal reduced in-situ methane emissions in a temperate rewetted bog grassland by a hundredfold. *Science of the Total Environment*, *721*, 137763. https://doi.org/10.1016/j.scitotenv.2020.137763

IPCC. (2014). *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*.

Jaatinen, K., Fritze, H., Laine, J., & Laiho, R. (2007). Effects of short- and long-term water-level drawdown on the populations and activity of aerobic decomposers in a boreal peatland. *Global Change Biology*, *13*(2), 491–510. https://doi.org/10.1111/j.1365-2486.2006.01312.x

Joabsson, A., Christensen, T. R., & Wallén, B. (1999). Vascular plant controls on methane emissions from northern peatforming wetlands. *Trends in Ecology and Evolution*, *14*(10), 385–388. https://doi.org/10.1016/S0169-5347(99)01649-3

Joosten, H. (2009). *The Global Peatland CO_2 Picture*. Peatland Status and drainage emissions in all countries of the world.

Juottonen, H., Galand, P. E., Tuittila, E. S., Laine, J., Fritze, H., & Yrjälä, K. (2005). Methanogen communities and Bacteria along an ecohydrological gradient in a northern raised bog complex. *Environmental Microbiology*, *7*(10), 1547–1557. https://doi.org/10.1111/j.1462-2920.2005.00838.x

Juottonen, H., Hynninen, A., Nieminen, M., Tuomivirta, T. T., Tuittila, E.-S., Nousiainen, H., Kell, D. K., Yrjälä, K., Tervahauta, A., & Fritze, H. (2012). Methane-cycling microbial communities and methane emission in natural and restored peatlands. *Applied and Environmental Microbiology*, *78*(17), 6386–6389. https://doi.org/10.1128/AEM.00261-12

Kang, X., Yan, L., Cui, L., Zhang, X., Hao, Y., Wu, H., Zhang, Y., Li, W., Zhang, K., Yan, Z., Li, Y., & Wang, J. (2018). Reduced carbon dioxide sink and methane source under extreme drought condition in an alpine peatland. *Sustainability*, *10*(11), 1–15. https://doi.org/10.3390/su10114285

Kiener, A., & Leisinger, T. (1983). Oxygen Sensitivity of Methanogenic Bacteria. *Systematic and Applied Microbiology*, *4*(3), 305–312. https://doi.org/10.1016/S0723-2020(83)80017-4

Killham, K., & Prosser, J. I. (2006). *The Prokaryotes* (M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Chleifer, & E. Stackebrandt (eds.); 3rd ed.). Springer-Verlag. https://doi.org/10.1007/978-3-662-13187-9

Kim, S.-Y., Lee, S.-H., Freeman, C., Fenner, N., & Kang, H. (2008). Comparative analysis of soil microbial communities and their responses to the short-term drought in bog, fen, and riparian wetlands. *Soil Biology and Biochemistry*, *40*(11), 2874–2880. https://doi.org/10.1016/j.soilbio.2008.08.004

Kirk, J. L., Beaudette, L. A., Hart, M., Moutoglis, P., Klironomos, J. N., Lee, H., & Trevors, J. T. (2004). Methods of studying soil microbial diversity. *Journal of Microbiological Methods*, *58*(2), 169–188. https://doi.org/10.1016/j.mimet.2004.04.006

Knief, C. (2015). Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on pmoA as molecular marker. *Frontiers in Microbiology*, *6*, 1–38. https://doi.org/10.3389/fmicb.2015.01346

Knorr, K.-H., & Blodau, C. (2009). Impact of experimental drought and rewetting on redox transformations and methanogenesis in mesocosms of a northern fen soil. *Soil Biology and Biochemistry*, *41*(6), 1187–1198. https://doi.org/10.1016/j.soilbio.2009.02.030

Knorr, K.-H., Glaser, B., & Blodau, C. (2008). Fluxes and 13C isotopic composition of dissolved carbon and pathways of methanogenesis in a fen soil exposed to experimental drought. *Biogeosciences Discussions*, *5*(2), 1319–1360. https://doi.org/10.5194/bgd-5-1319-2008

Koebsch, F., Winkel, M., Liebner, S., Liu, B., Westphal, J., Schmiedinger, I., Spitzy, A., Gehre, M., Jurasinski, G., Köhler, S., Unger, V., Koch, M., Sachs, T., & Böttcher, M. E. (2019). Sulfate deprivation triggers high methane production in a disturbed and rewetted coastal peatland. *Biogeosciences*, *16*(9), 1937–1953. https://doi.org/10.5194/bg-16-1937-2019

Komulainen, V. M., Tuittila, E.-S., Vasander, H., & Laine, J. (1999). Restoration of drained peatlands in southern Finland: Initial effects on vegetation change and CO2 balance. *Journal of Applied Ecology*, *36*(5), 634–648. https://doi.org/10.1046/j.1365-2664.1999.00430.x

Krause, S., van Bodegom, P. M., Cornwell, W. K., & Bodelier, P. L. E. (2014). Weak phylogenetic signal in physiological traits of methane-oxidizing bacteria. *Journal of Evolutionary Biology*, *27*(6), 1240–1247. https://doi.org/10.1111/jeb.12401

Krause, Sascha, Niklaus, P. A., Morcillo, S. B., Franke, M. M., Lüke, C., Reim, A., & Bodelier, P. L. E. (2015). Compositional and functional stability of aerobic methane consuming communities in drained and rewetted peat meadows. *FEMS Microbiology Ecology*, *91*(11), 1–11. https://doi.org/10.1093/femsec/fiv119

Krumholz, L. R., Hollenback, J. L., Roskes, S. J., & Ringelberg, D. B. (1995). Methanogenesis and methanotrophy within a Sphagnum peatland. *FEMS Microbiology Ecology*, *18*(3), 215–224. https://doi.org/10.1016/0168-6496(95)00061-4

Laine, J., Vasander, H., & Laiho, R. (1995). Long-Term Effects of Water Level Drawdown on the Vegetation of Drained Pine Mires in Southern Finland Author (s): Jukaine , Jukka Laine , Harri Vasander and Raija Laiho Source : Journal of Applied Ecology , Vol . 32 , No . 4 (Nov ., 1995), pp . 785-80. *Journal of Applied Ecology*, *32*, 785–802.

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

Liebner, S., Schwarzenbach, S. P., & Zeyer, J. (2012). Methane emissions from an alpine fen in central Switzerland. *Biogeochemistry*, *109*, 287–299. https://doi.org/10.1007/s10533-011-9629-4

Liebner, S., & Svenning, M. M. (2013). Environmental transcription of mmoX by methane-oxidizing Proteobacteria in a subarctic palsa peatland. *Applied and Environmental Microbiology*, *79*(2), 701–706. https://doi.org/10.1128/AEM.02292-12

Lu, Y., & Conrad, R. (2005). In situ stable isotope probing of methanogenic Archaea in the rice rhizosphere. *Science*, *309*(5737), 1088–1090. https://doi.org/10.1126/science.1113435

Lundin, L., Nilsson, T., Jordan, S., Lode, E., & Strömgren, M. (2017). Impacts of rewetting on peat, hydrology and water chemical composition over 15 years in two finished peat extraction areas in Sweden. *Wetlands Ecology and Management*, *25*, 405–419. https://doi.org/10.1007/s11273-016-9524-9

Luo, H., Sun, Z., Arndt, W., Shi, J., Friedman, R., & Tang, J. (2009). Gene order phylogeny and the evolution of methanogens. *PLoS ONE*, *4*(6), 2–6. https://doi.org/10.1371/journal.pone.0006069

Ma, K., & Lu, Y. (2011). Regulation of microbial methane production and oxidation by intermittent drainage in rice field soil. *FEMS Microbiology Ecology*, *75*, 446–456. https://doi.org/10.1111/j.1574-6941.2010.01018.x

McDonald, I. R., Bodrossy, L., Chen, Y., & Murrell, J. C. (2008). Molecular ecology techniques for the study of aerobic methanotrophs. *Applied and Environmental Microbiology*, *74*(5), 1305–1315. https://doi.org/10.1128/AEM.02233-07

McNicol, G., Knox, S. H., Guilderson, T. P., Baldocchi, D. D., & Silver, W. L. (2020). Where old meets new: An ecosystem study of methanogenesis in a reflooded agricultural peatland. *Global Change Biology*, *26*(2), 772–785. https://doi.org/10.1111/gcb.14916

Mitsch, W., & Gosselink, J. (2015). Wetlands. In *Wetlands* (5th ed.). Jon Wiley & Sons, Inc.

Moore, T. R., & Roulet, N. T. (1993). Methane flux: Water table relations in northern wetlands. *Geophysical Research Letters*, *20*(7), 587–590. https://doi.org/10.1029/93GL00208

Myhre, G., Shindell, D., Bréon, F.-M., Collins, W. D., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J.-F., Lee, D., Mendoza, B., Nakajima, T., Robock, a., Stephens, G., Takemura, T., & Zhan, H. (2013). IPCC AR5 (2013) Chapter 8: Anthropogenic and Natural Radiative Forcing. In *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. https://doi.org/10.1017/ CBO9781107415324.018

Op den Camp, H. J. M., Mohammadi, S. S., Pol, A., & Dunfield, P. F. (2018). Verrucomicrobial Methanotrophs. In M. G. Kalyuzhnaya & X.-H. Xing (Eds.), *Methane Biocatalysis: Paving the Way to Sustainability* (pp. 43–55). Springer International Publishing. https://doi.org/10.1007/978-3-319-74866-5_3

Pabinger, S., Rödiger, S., Kriegner, A., Vierlinger, K., & Weinhäusel, A. (2014). A survey of tools for the analysis of quantitative PCR (qPCR) data. *Biomolecular Detection and Quantification*, *1*, 23–33. https://doi.org/10.1016/j.bdq.2014.08.002

Pan, Y., Abell, G. C. J., Bodelier, P. L. E., Meima-Franke, M., Sessitsch, A., & Bodrossy, L. (2014). Remarkable Recovery and Colonization Behaviour of Methane Oxidizing Bacteria in Soil After Disturbance Is Controlled by Methane Source Only. *Microbial Ecology*, *68*, 259–270. https://doi.org/10.1007/s00248-014-0402-9

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

Peltoniemi, K., Laiho, R., Juottonen, H., Kiikkilä, O., Mäkiranta, P., Minkkinen, K., Pennanen, T., Penttilä, T., Sarjala, T., Tuittila, E.-S., Tuomivirta, T., & Fritze, H. (2015). Microbial ecology in a future climate: Effects of temperature and moisture on microbial communities of two boreal fens. *FEMS Microbiology Ecology*, *91*(7), 1–14. https://doi.org/10.1093/femsec/fiv062

Pinsonneault, A. J., Moore, T. R., & Roulet, N. T. (2016). Temperature the dominant control on the enzyme-latch across a range of temperate peatland types. *Soil Biology and Biochemistry*, *97*, 121–130. https://doi.org/10.1016/j.soilbio.2016.03.006

Pinto, A. J., & Raskin, L. (2012). PCR Biases Distort Bacterial and Archaeal Community Structure in Pyrosequencing Datasets. *PLoS ONE*, *7*(8), e43093. https://doi.org/10.1371/journal.pone.0043093

Postollec, F., Falentin, H., Pavan, S., Combrisson, J., & Sohier, D. (2011). Recent advances in quantitative PCR (qPCR) applications in food microbiology. *Food Microbiology*, *28*, 848–861. https://doi.org/10.1016/j.fm.2011.02.008

Quince, C., Lanzen, A., Davenport, R. J., & Turnbaugh, P. J. (2011). Removing Noise From Pyrosequenced Amplicons. *BMC Bioinformatics*, *12*. https://doi.org/10.1186/1471-2105-12-38

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

Ramette, A. (2009). Quantitative Community Fingerprinting Methods for Estimating the Abundance of Operational Taxonomic Units in Natural Microbial Communities. *Applied and Environmental Microbiology*, *75*(8), 2495–2505. https://doi.org/10.1128/AEM.02409-08

Reeburgh, W. S. (1980). Anaerobic methane oxidation: Rate depth distributions in Skan Bay sediments. *Earth and Planetary Science Letters*, *47*(3), 345–352. https://doi.org/10.1016/0012-821X(80)90021-7

Reim, A., Hernández, M., Klose, M., Chidthaisong, A., Yuttitham, M., & Conrad, R. (2017). Response of methanogenic microbial communities to desiccation stress in flooded and rain-fed paddy soil from Thailand. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.00785

Reumer, M., Harnisz, M., Lee, H. J., Reim, A., Grunert, O., Putkinen, A., Fritze, H., Bodelier, P.

L. E., & Ho, A. (2018). Impact of peat mining and restoration on methane turnover potential and methane-cycling microorganisms in a northern bog. *Applied and Environmental Microbiology*, *84*(3). https://doi.org/10.1128/AEM.02218-17

Rincon-Florez, V. A., Carvalhais, L. C., & Schenk, P. M. (2013). Culture-independent molecular tools for soil and rhizosphere microbiology. *Diversity*, *5*(3), 581–612. https://doi.org/10.3390/d5030581

Roden, E. E., & Wetzel, R. G. (2002). Kinetics of microbial Fe(III) oxide reduction in freshwater wetland sediments. *Limnology and Oceanography*, *47*(1), 198–211. https://doi.org/10.4319/lo.2002.47.1.0198

Roslev, P., & King, G. M. (1996). Regulation of methane oxidation in a freshwater wetland by water table changes and anoxia. *FEMS Microbiology Ecology*, *19*, 105–115. https://doi.org/10.1016/0168-6496(95)00084-4

Russel, H., Carita, F., Gavin, D., & Robert, W. (1993). Kinetic PCR Analysis: Real-time Monitoring of DNA Amplification Reactions. *Biotechnology*, *11*, 1026–1030. http://www.nature.com/naturebiotechnology

Saunois, M., R. Stavert, A., Poulter, B., Bousquet, P., G. Canadell, J., B. Jackson, R., A. Raymond, P., J. Dlugokencky, E., Houweling, S., K. Patra, P., Ciais, P., K. Arora, V., Bastviken, D., Bergamaschi, P., R. Blake, D., Brailsford, G., Bruhwiler, L., M. Carlson, K., Carrol, M., … Zhuang, Q. (2020). The global methane budget 2000-2017. *Earth System Science Data*, *12*(3), 1561–1623. https://doi.org/10.5194/essd-12-1561-2020

Schnürer, J., & Rosswall, T. (1982). Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Applied and Environmental Microbiology*, *43*(6), 1256–1261. https://doi.org/10.1128/aem.43.6.1256-1261.1982

Segarra, K. E. A., Schubotz, F., Samarkin, V., Yoshinaga, M. Y., Hinrichs, K.-U., & Joye, S. B. (2015). High rates of anaerobic methane oxidation in freshwater wetlands reduce potential atmospheric methane emissions. *Nature Communications*, *6*. https://doi.org/10.1038/ncomms8477

Segers, R. (1998). Methane production and methane consumption: a review of processes underlying wetland methane fluxes. *Biogeochemistry*, *41*, 23–51. https://doi.org/10.1023/A:1005929032764

Serrano-Silva, N., Sarria-Guzmán, Y., Dendooven, L., & Luna-Guido, M. (2014).

Methanogenesis and Methanotrophy in Soil: A Review. *Pedosphere*, *24*(3), 291–307. https://doi.org/10.1016/S1002-0160(14)60016-3

Shao, X., Sheng, X., Wu, M., Wu, H., & Ning, X. (2017). Methane production potential and emission at different water levels in the restored reed wetland of Hangzhou Bay. *PLoS ONE*, *12*(10), 1–13. https://doi.org/10.1371/journal.pone.0185709

Sikora, A., Detman, A., Chojnacka, A., & Blaszczyk, M. K. (2017). Anaerobic Digestion: I. A Common Process Ensuring Energy Flow and the Circulation of Matter in Ecosystems. II. A Tool for the Production of Gaseous Biofuels. In A. Jozala (Ed.), *Fermentation Processes*. https://doi.org/10.5772/64645

Smith, G. J., & Wrighton, K. C. (2019). Metagenomic Approaches Unearth Methanotroph Phylogenetic and Metabolic Diversity. *Current Issues in Molecular Biology*, *33*, 57–84. https://doi.org/10.21775/cimb.033.057

Smith, K. A., Ball, T., Conen, F., Dobbie, K. E., Massheder, J., & Rey, A. (2018). Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. *European Journal of Soil Science*, *69*, 10–20. https://doi.org/10.1111/ejss.12539

Sogin, M. L., Morrison, H. G., Huber, J. A., Welch, D. M., Huse, S. M., Neal, P. R., Arrieta, J. M., & Herndl, G. J. (2006). Microbial diversity in the deep sea and the underexplored “rare biosphere.” *Proceedings of the National Academy of Sciences*, *103*(32), 12115–12120. https://doi.org/10.1073/pnas.0605127103

Solaiman, Z. (2007). Measurement of Microbial Biomass and Activity in Soil. In A. Varma & R. Oelmüller (Eds.), *Advanced Techniques in Soil Microbiology* (Vol. 11, pp. 201–211). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-70865-0_13

Sousa, D. Z., Salvador, A. F., Ramos, J., Guedes, A. P., Barbosa, S., Stams, A. J. M., Alves, M. M., & Pereira, M. A. (2013). Activity and viability of methanogens in anaerobic digestion of unsaturated and saturated long-chain fatty acids. *Applied and Environmental Microbiology*, *79*(14), 4239–4245. https://doi.org/10.1128/AEM.00035-13

Tang, L. (2020). Exploring microbial worlds. *Nature Methods*, *17*(1), 22–22. https://doi.org/10.1038/s41592-019-0707-1

Tian, J., Shu, C., Chen, H., Qiao, Y., Yang, G., Xiong, W., Wang, L., Sun, J., & Liu, X. (2015). Response of archaeal communities to water regimes under simulated warming and drought conditions in Tibetan Plateau wetlands. *Journal of Soils and Sediments*, *15*, 179–188. https://doi.org/10.1007/s11368-014-0978-1

Tian, J., Zhu, Y., Kang, X., Dong, X., Li, W., Chen, H., & Wang, Y. (2012). Effects of drought on the archaeal community in soil of the Zoige wetlands of the Qinghai-Tibetan plateau. *European Journal of Soil Biology*, *52*, 84–90. https://doi.org/10.1016/j.ejsobi.2012.07.003

Timmers, P. H. A., Suarez-Zuluaga, D. A., van Rossem, M., Diender, M., Stams, A. J., & Plugge, C. M. (2016). Anaerobic oxidation of methane associated with sulfate reduction in a natural freshwater gas source. *The ISME Journal*, *10*, 1400–1412. https://doi.org/10.1038/ismej.2015.213

Timmers, P. H. A., Welte, C. U., Koehorst, J. J., Plugge, C. M., Jetten, M. S. M., & Stams, A. J. M. (2017). Reverse Methanogenesis and Respiration in Methanotrophic Archaea. *Archaea*, *2017*, 1–23. https://doi.org/10.1155/2017/1654237

Tuittila, E.-S., Komulainen, V.-M., Vasander, H., Nykanen, H., Martikainen, P. J., & Laine, J. (2000). Methane dynamics of a restored cut-away peatland. *Global Change Biology*, *6*(5), 569–581. https://doi.org/10.1046/j.1365-2486.2000.00341.x

Turetsky, M. R., Kotowska, A., Bubier, J., Dise, N. B., Crill, P., Hornibrook, E. R. C., Minkkinen, K., Moore, T. R., Myers-Smith, I. H., Nykänen, H., Olefeldt, D., Rinne, J., Saarnio, S., Shurpali, N., Tuittila, E.-S., Waddington, J. M., White, J. R., Wickland, K. P., & Wilmking, M. (2014). A synthesis of methane emissions from 71 northern, temperate, and subtropical wetlands. *Global Change Biology*, *20*(7), 2183–2197. https://doi.org/10.1111/gcb.12580

Urbanová, Z., & Bárta, J. (2016). Effects of long-term drainage on microbial community composition vary between peatland types. *Soil Biology and Biochemistry*, *92*, 16–26. https://doi.org/10.1016/j.soilbio.2015.09.017

Urbanová, Z., & Bárta, J. (2020). Recovery of methanogenic community and its activity in long-term drained peatlands after rewetting. *Ecological Engineering*, *150*. https://doi.org/10.1016/j.ecoleng.2020.105852

Urbanová, Z., Bárta, J., & Picek, T. (2013). Methane Emissions and Methanogenic Archaea on Pristine, Drained and Restored Mountain Peatlands, Central Europe. *Ecosystems*, *16*(4), 664–677. https://doi.org/10.1007/s10021-013-9637-4

Urich, T., Lanzén, A., Qi, J., Huson, D. H., Schleper, C., & Schuster, S. C. (2008). Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. *PLoS ONE*, *3*(6). https://doi.org/10.1371/journal.pone.0002527

van Bodegom, P. M., Stams, F., Mollema, L., Boeke, S., & Leffelaar, P. (2001). Methane oxidation and competition for oxygen in the rice rhizosphere. *Applied and Environmental Microbiology*, *67*(8). https://doi.org/10.1128/AEM.67.8.3586–3597.2001

van Dorst, J., Bissett, A., Palmer, A. S., Brown, M., Snape, I., Stark, J. S., Raymond, B., McKinlay, J., Ji, M., Winsley, T., & Ferrari, B. C. (2014). Community fingerprinting in a sequencing world. *FEMS Microbiology Ecology*, *89*(2), 316–330. https://doi.org/10.1111/1574-6941.12308

van Kruistum, H., Bodelier, P. L. E., Ho, A., Meima-Franke, M., & Veraart, A. J. (2018). Resistance and Recovery of Methane-Oxidizing Communities Depends on Stress Regime and History; A Microcosm Study. *Frontiers in Microbiology*, *9*(July), 1–14. https://doi.org/10.3389/fmicb.2018.01714

Vanselow-Algan, M., Schmidt, S. R., Greven, M., Fiencke, C., Kutzbach, L., & Pfeiffer, E.-M. (2015). High methane emissions dominated annual greenhouse gas balances 30 years after bog rewetting. *Biogeosciences*, *12*(14), 4361–4371. https://doi.org/10.5194/bg-12-4361-2015

Vanwonterghem, I., Evans, P. N., Parks, D. H., Jensen, P. D., Woodcroft, B. J., Hugenholtz, P., & Tyson, G. W. (2016). Methylotrophic methanogenesis discovered in the archaeal phylum Verstraetearchaeota. *Nature Microbiology*, *1*, 1–9. https://doi.org/10.1038/nmicrobiol.2016.170

Waddington, J. M., & Day, S. M. (2007). Methane emissions from a peatland following restoration. *Journal of Geophysical Research: Biogeosciences*, *112*(G3), 1–11. https://doi.org/10.1029/2007JG000400

Waddington, J. M., Roulet, N. T., & Swanson, R. V. (1996). Water table control of CH4 emission enhancement by vascular plants in boreal peatlands. *Journal of Geophysical Research Atmospheres*, *101*(D17), 22775–22785. https://doi.org/10.1029/96jd02014

Wang, H., Weil, M., Zak, D., Münch, D., Günther, A., Jurasinski, G., & Urich, T. (2020). Temporal and spatial dynamics of peat microbiomes in drained and rewetted soils of three temperate peatlands. *BioRxiv Preprint*. https://doi.org/10.1101/2020.02.16.951285

Wegener, G., Kellermann, M. Y., & Elvert, M. (2016). Tracking activity and function of microorganisms by stable isotope probing of membrane lipids. *Current Opinion in Biotechnology*, *41*, 43–52. https://doi.org/10.1016/j.copbio.2016.04.022

Weil, M., Wang, H., Bengtsson, M., Köhn, D., Günther, A., Jurasinski, G., Couwenberg, J., Negassa, W., Zak, D., & Urich, T. (2020). Long-term rewetting of three formerly drained peatlands drives congruent compositional changes in pro- and eukaryotic soil microbiomes through environmental filtering. *Microorganisms*, *8*. https://doi.org/10.3390/microorganisms8040550

Wemheuer, B., Wemheuer, F., & Daniel, R. (2012). RNA-based assessment of diversity and composition of active archaeal communities in the german bight. *Archaea*, *2012*. https://doi.org/10.1155/2012/695826

Wen, X., Unger, V., Jurasinski, G., Koebsch, F., Horn, F., Rehder, G., Sachs, T., Zak, D., Lischeid, G., Knorr, K. H., Böttcher, M. E., Winkel, M., Bodelier, P. L. E., & Liebner, S. (2018). Predominance of methanogens over methanotrophs in rewetted fens characterized by high methane emissions. *Biogeosciences*, *15*(21), 6519–6536. https://doi.org/10.5194/bg-15-6519-2018

Williams, E. L., Bagg, E. A. L., Mueller, M., Vandrovcova, J., Aitman, T. J., & Rumsby, G. (2015). Performance evaluation of Sanger sequencing for the diagnosis of primary hyperoxaluria and comparison with targeted next generation sequencing. *Molecular Genetics & Genomic Medicine*, *3*(1), 69–78. https://doi.org/10.1002/mgg3.118

Wilson, D., Farrell, C. A., Fallon, D., Moser, G., Müller, C., & Renou-Wilson, F. (2016). Multiyear greenhouse gas balances at a rewetted temperate peatland. *Global Change Biology*, *22*(12), 4080–4095. https://doi.org/10.1111/gcb.13325

Wu, M. L., Ettwig, K. F., Jetten, M. S. M., Strous, M., Keltjens, J. T., & Niftrik, L. van. (2011). A new intra-aerobic metabolism in the nitrite-dependent anaerobic methane-oxidizing bacterium Candidatus 'Methylomirabilis oxyfera.' *Biochemical Society Transactions*, *39*(1), 243–248. https://doi.org/10.1042/BST0390243

Yavitt, J. B., Lang, G. E., & Downey, D. M. (1988). Potential methane production and methane oxidation rates in peatland ecosystems of the Appalachian Mountains, United States. *Global Biogeochemical Cycles*, *2*(3), 253–268. https://doi.org/10.1029/GB002i003p00253

Zak, D., & Gelbrecht, J. (2007). The mobilisation of phosphorus, organic carbon and ammonium in the initial stage of fen rewetting (a case study from NE Germany). *Biogeochemistry*, *85*, 141–151. https://doi.org/10.1007/s10533-007-9122-2

Zak, D., Roth, C., Unger, V., Goldhammer, T., Fenner, N., Freeman, C., & Jurasinski, G. (2019). Unraveling the Importance of Polyphenols for Microbial Carbon Mineralization in Rewetted Riparian Peatlands. *Frontiers in Environmental Science*, *7*(October), 1–14. https://doi.org/10.3389/fenvs.2019.00147

Zhu, B., van Dijk, G., Fritz, C., Smolders, A. J. P., Pol, A., Jetten, M. S. M., & Ettwig, K. F. (2012). Anaerobic Oxidization of Methane in a Minerotrophic Peatland: Enrichment of Nitrite-Dependent Methane-Oxidizing Bacteria. *Applied and Environmental Microbiology*, *78*(24), 8657–8665. https://doi.org/10.1128/AEM.02102-12

A. Predominance of methanogens over methanotrophs in rewetted fens with high methane emissions

Abstract

The rewetting of drained peatlands alters peat geochemistry and often leads to sustained elevated methane emission. Although this methane is produced entirely by microbial activity, the distribution and abundance of methane-cycling microbes in rewetted peatlands, especially in fens, is rarely described. In this study, we compare the community composition and abundance of methane-cycling microbes in relation to peat porewater geochemistry in two rewetted fens in northeastern Germany, a coastal brackish fen and a freshwater riparian fen, with known high methane fluxes. We utilized 16S rRNA high-throughput sequencing and quantitative polymerase chain reaction (qPCR) on 16S rRNA, *mcrA*, and *pmoA* genes to determine microbial community composition and the abundance of total bacteria, methanogens, and methanotrophs. Electrical conductivity (EC) was more than 3 times higher in the coastal fen than in the riparian fen, averaging 5.3 and 1.5 mS cm^{-1}, respectively. Porewater concentrations of terminal electron acceptors (TEAs) varied within and among the fens. This was also reflected in similarly high intra- and inter-site variations of microbial community composition. Despite these differences in environmental conditions and electron acceptor availability, we found a low abundance of methanotrophs and a high abundance of methanogens, represented in particular by Methanosaetaceae, in both fens. This suggests that rapid (re)establishment of methanogens and slow (re)establishment of methanotrophs contributes to prolonged increased methane emissions following rewetting.

Introduction

Rewetting is a technique commonly employed to restore ecological and biogeochemical functioning of drained fens. However, while rewetting may reduce carbon dioxide (CO_2) emissions (Wilson et al., 2016), it often increases methane (CH_4) emissions in peatlands that remain inundated following rewetting. On a 100-year timescale, CH_4 has a global warming potential 28 times stronger than CO_2 (Myhre et al., 2013), and the factors that contribute to the magnitude and duration of increased emissions are still uncertain (Joosten et al., 2015; Abdalla et al., 2016). Thus, elucidating the dynamics of post-rewetting CH_4 exchange is of strong interest for both modeling studies and peatland management projects (Abdalla et al., 2016). Although a recent increase in rewetting projects in Germany and other European countries has prompted a number of studies of methane cycling in rewetted peatlands (e.g., Jerman et al., 2009; Hahn-Schöfl et al., 2011; Urbanová et al., 2013; Hahn et al., 2015; Vanselow-Algan et al., 2015; Zak et al., 2015; Emsens et al., 2016; Putkinen et al., 2018), the post-rewetting distribution and abundance of methane-cycling microbes in rewetted fens has seldom been examined (but see Juottonen et al., 2012; Urbanová et al., 2013; Putkinen et al., 2018).

Peat CH_4 production and release is governed by a complex array of interrelated factors including climate, water level, plant community, nutrient status, site geochemistry, and the activity of microbes (i.e., bacteria and archaea) that use organic carbon as an energy source (Segers, 1998; Abdalla et al., 2016). To date, the vast majority of studies in rewetted fens have focused on quantifying CH_4 emission rates in association with environmental variables such as water level, plant community, and aspects of site geochemistry (Abdalla et al., 2016). Site geochemistry indeed plays an important role for methanogenic communities, as methanogenesis is suppressed in the presence of thermodynamically more favorable terminal electron acceptors (TEAs, Blodau 2011). Due to a smaller pool of more favorable electron acceptors and high availability of organic carbon substrates, organic-rich soils such as peat rapidly establish methanogenic conditions post-rewetting (Segers, 1998; Keller and Bridgham 2007; Knorr and Blodau 2009). Despite their decisive role as producers (i.e., methanogens) and consumers (i.e., methanotrophs) of CH_4 (Conrad 1996), only a few studies have combined a characterization of the CH_4-cycling microbial community, site geochemistry, and observed trends in CH_4 production. Existing studies have been conducted in oligotrophic and mesotrophic boreal fens (e.g., Juottonen et al., 2005, 2012; Yrjälä et al., 2011), alpine fens (e.g., Liebner et al., 2012; Urbanová et al., 2013; Cheema et al., 2015; Franchini et al., 2015), subarctic fens (Liebner et al., 2015), and incubation experiments (e.g., Jerman et al., 2009; Knorr and Blodau, 2009; Urbanová et al., 2011; Emsens et al., 2016). Several studies on CH_4-cycling microbial communities have been conducted in minerotrophic temperate fens (e.g., Cadillo-Quiroz et al., 2008; Liu et al., 2011; Sun et al., 2012; Zhou et al., 2017), but these sites were not subject to drainage or rewetting. Direct comparisons of *in situ* abundances of methanogens and methanotrophs in drained versus rewetted fens are scarce (Juottonen et al., 2012; Putkinen et al., 2018), and the studied sites, so far, are nutrient-poor fens with acidic conditions.

While studies of nutrient-poor and mesotrophic boreal fens have documented post-rewetting CH$_4$ emissions comparable to or lower than at pristine sites (Komulainen et al., 1998; Tuittila et al., 2000; Juottonen et al., 2012), studies of temperate nutrient-rich fens have reported post-flooding CH$_4$ emissions dramatically exceeding emissions in pristine fens (e.g., Augustin and Chojnicki, 2008; Hahn et al., 2015). These high emissions typically occur together with a significant dieback in vegetation, a mobilization of nutrients and electron acceptors in the upper peat layer, and increased availability of dissolved organic matter (Zak and Gelbrecht, 2007; Hahn-Schöfl et al., 2011; Hahn et al., 2015; Jurasinski et al., 2016). High CH$_4$ fluxes may continue for decades following rewetting, even in bogs (Vanselow-Algan et al., 2015). Hence, there is an urgent need to characterize CH$_4$-cycling microbial communities and geochemical conditions in rewetted minerotrophic fens. In this study, we therefore examined microbial community composition and abundance in relation to post-flooding geochemical conditions in two rewetted fens in northeastern Germany. In both fens, CH$_4$ emissions increased dramatically after rewetting, to over 200 g C m^{-2} a^{-1} (Augustin and Chojnicki, 2008; Hahn-Schöfl et al., 2011; Hahn et al., 2015; Jurasinski et al., 2016). Average annual CH$_4$ emissions have decreased in both fens since the initial peak (Franz et al., 2016; Jurasinski et al., 2016). Nevertheless, fluxes remained higher than under pre-flooding conditions (ibid.) and higher than in pristine fens (Urbanová et al., 2013; Minke et al., 2016). In the Hütelmoor in 2012, average CH$_4$ emissions during the growing season were 40 g m^{-2} (Koebsch et al., 2015). In Zarnekow, average CH$_4$ emissions were 40 g m^{-2} for the year 2013 (Franz et al., 2016). In comparison, a recent review paper (Abdalla et al., 2016) estimated an average flux of 12 ± 21 g C m^{-2} a^{-1} for pristine peatlands.

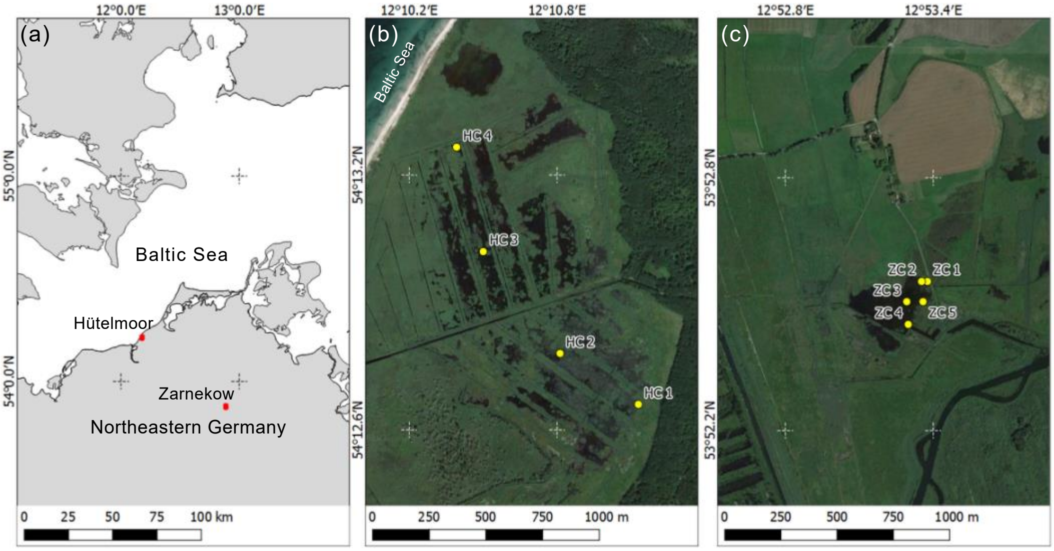


Figure A-1 – Location of study sites in northeastern Germany (a) and sampling locations within sites (b) Hütelmoor and (c) Zarnekow. Maps (b) and (c) are drawn to the same scale. Image source: (a) QGIS; (b) and (c) Google Earth via QGIS OpenLayers Plugin. Imagery date 9 August 2015.

We expected patterns in microbial community composition would reflect the geochemical conditions of the two sites and hypothesized a high abundance of methanogens relative to methanotrophs in both fens. We also expected acetoclastic methanogens, which typically thrive in nutrient-rich fens (Kelly et al., 1992; Galand et al., 2005), to dominate the methanogenic community in both fens.

Materials and methods

Study sites

The nature reserve Heiligensee and Hütelmoor ("Hütelmoor" in the following; approx. 540 ha; 54°12′36.66″ N, 12°10′34.28″ E) is a coastal, mainly minerotrophic fen complex in Mecklenburg-Vorpommern (NE Germany) that is separated from the Baltic Sea by a narrow (~ 100 m and less) dune dike (Fig. A-1a and b). The climate is temperate in the transition zone between maritime and continental, with an average annual temperature of 9.1 °C and an average annual precipitation of 645 mm (data derived from the grid product of the German Weather Service, reference climate period 1981–2010). Episodic flooding from storm events delivers sediment and brackish water to the site (Weisner and Schernewski, 2013). The vegetation is a mixture of salt-tolerant macrophytes, with dominant to semi-dominant stands of *Phragmites australis*, *Bolboschoenus maritimus*, *Carex acutiformis*, and *Schoenoplectus tabernaemontani*. The dominating plants are interspersed with open water bodies that are colonized by Ceratophyllum demersum in summer (Koch et al., 2017). Intense draining and land amelioration practices began in the 1970s, which lowered the water level to 1.6 m below ground surface and caused aerobic decomposition and concomitant degradation of the peat (Voigtländer et al., 1996). The upper peat layer varies in depth between 0.6 and 3 m and is highly degraded, reaching up to H10 on the von Post humification scale (Hahn et al., 2015). Active draining ended in 1992, but dry conditions during summertime kept the water table well below ground surface (Schönfeld-Bockholt et al., 2008; Koebsch et al., 2013) until concerns of prolonged aerobic peat decomposition prompted the installation of a weir in 2009 at the outflow of the catchment (Weisner and Schernewski, 2013). After installation of the weir, the site has been fully flooded year-round with an average water level of 0.6 m above the peat surface, and annual average CH_4 flux increased ~186-fold from 0.0014 ± 0.0006 to 0.26 ± 0.06 kg CH_4 m^{-2} a^{-1} (Hahn et al., 2015).

The study site polder Zarnekow ("Zarnekow" in the following; approx. 500 ha; 53°52′31.10′ N, 12°53′19.60″ E) is situated in the valley of the river Peene in Mecklenburg-Vorpommern (NE Germany, Fig. A-1a and c). The climate is slightly more continental compared to the Hütelmoor, with a mean annual precipitation of 544 mm and a mean annual temperature of 8.7°C (German Weather Service, meteorological station Teterow, 24 km southwest of the study site; reference period 1981–2010). The fen can be classified as a river valley mire system consisting of spring mires, wider percolation mires, and flood mires along the river Peene. Drainage and low-intensity agricultural use began in the 18th century when land use changed to pastures and grassland. This was intensified by active pumping in the mid-1970s. Due to land subsidence of several decimeters, after rewetting (October 2004) the water table depth increased to 0.1–0.5 m above the peat surface. The upper horizon is highly decomposed (0–0.3 m), followed by moderately decomposed peat to a depth of 1 m and a deep layer of slightly decomposed peat up to a maximum depth of 10 m. The open water bodies are densely colonized by *Ceratophyllum* spp. and *Typha latifolia* is the dominant emergent macrophyte (Steffenhagen et al., 2012). Following flooding, CH_4 flux rates increased to ~ 0.21 kg m^{-2} a^{-1} (Augustin and Chojnicki, 2008). No pre-rewetting CH_4 flux data were available for the Zarnekow site, but published CH_4 flux rates of representative drained fens from the same region have been shown to be negligible, and many were CH_4 sinks (Augustin et al., 1998).

Collection and analysis of peat cores and porewater samples

Peat and porewater samples were collected at four different locations (n = 4) in Hütelmoor (October 2014) and at five locations (n = 5) in Zarnekow (July 2015) and spanned a distance of 1200 and 250 m, respectively, to cover the whole lateral extension at each site (Fig. A-1b and c). Sampling depths in the Hütelmoor were 0–5, 5–10, 10–20, 20–30, 30–40, and 40–50 cm below the peat surface, except for core numbers 1 and 4 where samples could only be obtained up to a depth of 10–20 and 30–40cm, respectively. Sampling depths in Zarnekow were 0–5, 25–30, and 50–55 cm below the peat surface. Previous work at Zarnekow has revealed little variation in peat properties with depth (e.g., Zak and Gelbrecht 2007); hence, a lower depth resolution in Zarnekow cores (ZCs) was chosen for this study. Peat cores were collected with a Perspex liner (ID: 60mm, Hütelmoor) and a peat auger (Zarnekow). In order to minimize oxygen contamination, the outer layer of the peat core was omitted. Subsamples for molecular analysis were immediately packed in 15 mL sterile Falcon tubes and stored at -80°C until further processing.

Pore waters in the Hütelmoor were collected with a stainless-steel push-point sampler attached to a plastic syringe to recover the samples from 10cm depth intervals. Samples were immediately filtered with 0.45 µm membrane, sterile, disposable syringe filters. Pore waters in Zarnekow were sampled with permanently installed dialysis samplers consisting of slotted polypropylene (PP) pipes (length: 636 mm, ID: 34 mm) surrounded with 0.22 µm polyethersulfone membrane. The

PP pipes were fixed at distinct peat depths (surface level, 20 and 40cm depth) and connected with PP tubes (4 × 6 mm ID × AD). Water samples were drawn out from the dialysis sampler pipes with a syringe through the PP tube. Due to practical restrictions in accessibility and sampling, permanent dialysis samplers could not be installed at the desired locations in the Hütelmoor, resulting in the different sampling techniques described above.

At both sites, electrical conductivity (EC), dissolved oxygen (DO), and pH were measured immediately after sampling (Sentix 41 pH probe and a TetraCon 325 conductivity measuring cell attached to a WTW multi 340i hand-held; WTW, Weilheim). In this paper, EC is presented and was not converted to salinity (i.e., psu), as a conversion would be imprecise for brackish waters. A simplified equation for conversion can be found in Schemel (2001). Headspace CH_4 concentrations of porewater samples were measured with an Agilent 7890A gas chromatograph (Agilent Technologies, Germany) equipped with a flame ionization detector and a Carboxen PLOT Capillary Column or HP-Plot Q (Porapak-Q) column. The measured headspace CH_4 concentration was then converted into a dissolved CH_4 concentration using the temperature-corrected solubility coefficient (Wilhelm et al., 1977). Isotopic composition of dissolved CH_4 for Hütelmoor was analyzed using the gas-chromatography–combustion technique (GC-C) and the gas-chromatography–high-temperature-conversion technique (GC-HTC). The gas was directly injected in a gas chromatograph (Agilent 7890A), CH_4 was quantitatively converted to CO_2, and the $\delta^{13}C$ values were then measured with the isotope ratio mass spectrometer MAT-253 (Thermo Finnigan, Germany). The $\delta^{13}C$ of dissolved CH_4 in Zarnekow was analyzed using a laser-based isotope analyzer equipped with a small sample isotope module for analyses of discrete gas samples (cavity ring-down spectroscopy, CRDS; Picarro G2201-I, Santa Clara, CA, USA). Calibration was carried out before, during, and after analyses using certified standards of known isotopic composition (obtained from Isometric Instruments, Victoria, BC, Canada; and from Westfalen AG, Münster, Germany). Reproducibility of results was typically ±1 ‰. In the presence of high concentrations of hydrogen sulfide interfering with laser-based isotope analysis, samples were treated with iron(III) sulfate to oxidize and/or precipitate sulfide. For both sites, sulfate and nitrate concentrations were analyzed by ion chromatography (IC, Thermo Fisher Scientific Dionex) using an IonPac AS-9-HC 4 column, partly after dilution of the sample. Dissolved metal concentrations were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES, iCAP 6300 DUO, Thermo Fisher Scientific). Accuracy and precision were routinely checked with a certified CASS standard as previously described (Kowalski et al., 2012). For the incubation experiments, peat cores were collected from Zarnekow in March 2012 using a modified Kajak

Corer with a plexiglass tube. The intact cores were placed in a cool box and immediately transported to the Leibniz Institute of Freshwater Ecology and Inland Fisheries in Berlin, where they were sectioned into a total of 12 samples. Fresh, surficial organic sediment (0–10 cm depth, 6 individual samples) was separated from the bulk peat (10–20 cm depth, 6 individual samples) and the samples were placed in 60 mL plastic cups. The cups were filled completely and closed with air-tight caps to minimize oxygen contamination. The samples were then express-shipped (< 24 h) to the lab at the Netherlands Institute of Ecology for immediate processing and analysis. For CH_4 production incubations, 5 g of material and 10 mL of nitrogen (N_2)-flushed MilliQ water were weighed into three (n = 3) 150 mL flasks for both surficial organic sediment and bulk peat. The flasks were capped with rubber stoppers, flushed with N_2 for approximately 1 h, and then incubated stationarily at 20 °C in the dark. For CH_4 oxidation incubations, 5 g of fresh material and 10 mL of MilliQ water were weighed into three 150 mL flasks for both surficial organic sediment and bulk peat. The flasks were capped with rubber stoppers and 1.4 mL of pure CH_4 was added to obtain a headspace CH_4 concentration of approximately 10 000 ppm. Incubations were performed in the dark at 20 °C on a gyratory shaker (120 rpm). For all incubations, headspace CH_4 concentration was determined using a gas chromatograph equipped with a flame ionization detector on days 1, 3, 5, and 8 of the incubation. Potential CH_4 production and oxidation rates were determined by linear regression of CH_4 concentration over all sampling times.

Gene amplification and phylogenetic analysis

Genomic DNA was extracted from 0.2–0.3 g of duplicates of peat soil per sample using an EurX GeneMATRIX soil DNA Purification Kit (Roboklon, Berlin, Germany). DNA concentrations were quantified with a Nanophotometer P360 (Implen GmbH, Munich, Germany) and Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Darmstadt, Germany). Polymerase chain reaction (PCR) amplification of bacterial and archaeal 16S rRNA genes was performed using the primer combination of S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 (Herlemann et al., 2011) and S-D-Arch-0349-a-S-17/S-D-Arch-0786-a-A-20 (Takai and Horikoshi 2000), respectively, with barcodes contained in the 5′ end. The PCR mix contained 1× PCR buffer (Tris • Cl, KCl, $(NH_4)2SO_4$, 15 mM $MgCl_2$; pH 8.7) (QIAGEN, Hilden, Germany), 0.5 µM of each primer (Biomers, Ulm, Germany), 0.2 mM of each deoxynucleoside (Thermo Fisher Scientific, Darmstadt, Germany), and 0.025 U μL^{-1} hot start polymerase (QIAGEN, Hilden, Germany). PCR samples were kept at 95 °C for 5 min to denature the DNA, with amplification proceeding for 40 cycles at 95 °C for 1 min, 56 °C for 45s, and 72°C for 90s; a final extension of 10min at 72°C was added to ensure complete amplification. PCR products were purified with a Hi Yield Gel/PCR

DNA fragment extraction kit (Süd-Laborbedarf, Gauting, Germany). To reduce amplification bias, PCR products of three individual runs per sample were combined. PCR products of different samples were pooled in equimolar concentrations and compressed to a final volume of 10 µL with a concentration of 200 ng μL^{-1} in a vacuum centrifuge Concentrator Plus (Eppendorf, Hamburg, Germany).

Illumina sequencing was performed by GATC Biotech AG using 300 bp paired-end mode and a 20 % PhiX Control v3 library to counteract the effects of low-diversity sequence libraries. Raw data were demultiplexed using an own script based on CutAdapt (Martin, 2011). Ambiguous nucleotides at sequence ends were trimmed and a 10 % mismatch was allowed for primer identification, whereas barcode sequences needed to be present without any mismatches and with a minimum Phred score of Q25 for each nucleotide. After sorting, overlapping paired-end reads were merged using PEAR (Q25, p. 0.0001, v20) (Zhang et al., 2014). The orientation of the merged sequences was standardized according to the barcode information obtained from demultiplexing. Low-quality reads were removed using Trimmomatic (SE, LEADING Q25, TRAILING Q25, SLIDINGWINDOW 5 : 25; MINLEN 200) (Bolger et al., 2014). Chimeric sequences were removed using USEARCH 6.1 and the QIIME script identify_chimeric_seqs.py (Caporaso et al., 2010). Preprocessed sequences were taxonomically assigned to operational taxonomic units (OTUs) at a nucleotide sequence identity of 97 % using QIIME's pick_open_reference_otus.py script and the GreenGenes database 13.05 (McDonald et al., 2012) as reference. The taxonomic assignment of representative sequences was further checked for correct taxonomical classification by phylogenetic tree calculations in the ARB environment referenced against the SILVA database version 119 (Quast et al., 2013). The resulting OTU table was filtered for singletons, for OTUs assigned to chloroplasts or mitochondria, and for low-abundance OTUs (below 0.2 % within each sample). Archaeal and bacterial samples were processed separately while only OTUs that were assigned to the respective domain were considered for further analysis. For archaea, a total of 6 844 177 valid sequences were obtained, ranging from 60 496 to 398 660 in individual samples. These sequences were classified into 402 OTUs. For bacteria, a total of 2 586 148 valid sequences were obtained, ranging from 22 826 to 164 916 in individual samples. These sequences were classified into 843 OTUs. The OTU tables were then collapsed at a higher taxonomic level to generate the bubble plots. The 16S rRNA gene sequence data have been deposited at NCBI under the BioProject PRJNA356778. The Hütelmoor sequence read archive accession numbers are

SRR5118134-SRR5118155 for bacterial and SRR5119428-SRR5119449 for archaeal sequences. The Zarnekow accession numbers are SRR6854018-SRR6854033 and SRR6854205-SRR6854220 for bacterial and archaeal sequences, respectively.

qPCR analysis

Quantitative polymerase chain reaction (qPCR) for the determination of methanotrophic and methanogenic functional gene copy numbers and overall bacterial 16S rRNA gene copy numbers was performed via SYBR Green assays on a Bio-Rad CFX instrument (Bio-Rad, Munich, Germany) with slight modifications according to Liebner et al. (2015). The functional methanotrophic *pmoA* gene was amplified with the primer combination A189F/Mb661 (Kolb et al., 2003) suitable for detecting all known aerobic methanotrophic Proteobacteria. Annealing was done at 55°C after a seven-cycle-step touchdown starting at 62°C. The functional methanogenic *mcrA* gene was amplified with the *mlas* and *mcrA*-rev primer pair (Steinberg and Regan 2009), with annealing at 57 °C. The bacterial 16S rRNA gene was quantified with the primers Eub341F/Eub534R according to Degelmann et al. (2010), with annealing at 58 °C. Different DNA template concentrations were tested prior to the qPCR runs to determine optimal template concentration without inhibitions through co-extracts. The 25 µL reactions contained 12.5 µL of iTaq Universal SYBR Green Supermix (Bio-Rad, Munich, Germany), 0.25 µM concentrations of the primers, and 5µL of DNA template. Data acquisition was always done at 80 °C to avoid quantification of primer dimers. The specificity of each run was verified through melt-curve analysis and gel electrophoresis. Only runs with efficiencies between 82 % and 105 % were used for further analysis.

Measurements were performed in duplicates. The ratio of methanogens to methanotrophs was determined based on gene abundances of *mcrA* and *pmoA*. The marker gene for the soluble monooxygenase, *mmoX*, was neglected due to the absence of *Methylocella* in the sequencing data (Fig. A-4).

Data visualization and statistical analysis

All data visualization and statistical analysis were done in R (R Core Team, 2017). The taxonomic relative abundances across samples were visualized through bubble plots with the R package ggplot2 (Wickham, 2009). Differences in microbial community composition were visualized with two-dimensional non-metric multidimensional scaling (NMDS) based on Bray–Curtis distances. The NMDS ordinations were constructed using R package vegan (Oksanen et al., 2017). An environmental fit was performed on the ordinations to determine the measured geochemical parameters that may influence community composition. The geochemical data were fitted to the ordinations as vectors with a significance of $p < 0.05$. Depth profiles were constructed with the porewater geochemical data, as well as with the microbial abundances, to elucidate depth-wise trends and assess whether differences in microbial community and abundances among the two fens are related to differences in their respective geochemistry.

Results

Environmental characteristics and site geochemistry

The two rewetted fens varied substantially in their environmental characteristics (e.g., proximity to the sea) and porewater geochemistry (Fig. A-2, Tables A-1 and A-2). EC was more than 3 times higher in Hütelmoor than in Zarnekow, averaging 5.3 and 1.5 mS cm^{-1}, respectively. Mean values of pH were approximately neutral (6.5 to 7.0) in the upper peat profile and comparable in both fens until a depth of about 30 cm where pH decreased to ~ 6 in the Hütelmoor. Concentrations of the TEAs nitrate and sulfate were lower in Zarnekow and near zero in the pore water at all depths, while nitrate and sulfate were abundalnt in the upper and lower peat profile in Hütelmoor at ~ 1.5 to 3.0 mM and ~ 4 to 20 mM, respectively (Fig. A-2). Iron concentrations were higher in the Hütelmoor pore water, while manganese concentrations were higher in Zarnekow pore water. Dissolved oxygen concentrations in the upper peat profile (i.e., 0 to 25cm depths) were much higher in Hütelmoor than in Zarnekow (Fig. A-2). Here DO concentrations averaged ~ 0.25 mM until a depth of 15 cm at which they dropped sharply, reaching concentrations slightly below 0.05 mM at 25 cm. In Zarnekow, DO concentrations did not exceed 0.1 mM and varied little with depth. Regarding geochemical conditions, Hütelmoor core (HC) 1 differed from all other Hütelmoor cores and was more similar to Zarnekow cores. In HC 1 – the core taken nearest to potential freshwater sources (Fig. A-1b) – pore water EC and DO concentrations were lower while pH was slightly higher than in all other Hütelmoor cores. Moreover, this was the only Hütelmoor core where nitrate concentrations were below the detection limit (0.001 mM) (Fig. A-2). In all cores we found high concentrations of dissolved CH_4 that varied within and among fens and were slightly higher in Zarnekow pore water. Stable isotope ratios of $\partial^{13}C\text{-}CH_4$ (Fig. A-2) in the upper peat (approx. −59 ‰) suggest a predominance of acetoclastic methanogenesis, with a shift to hydrogenotrophic methanogenesis around −65 ‰ in the lower peat profile. Additionally, the observed shifts toward less negative $\partial^{13}C\text{-}CH_4$ values in the upper peat layer, as in HC 1 and HC 2, could indicate partial oxidation of CH_4 occurred (Chasar et al., 2000).

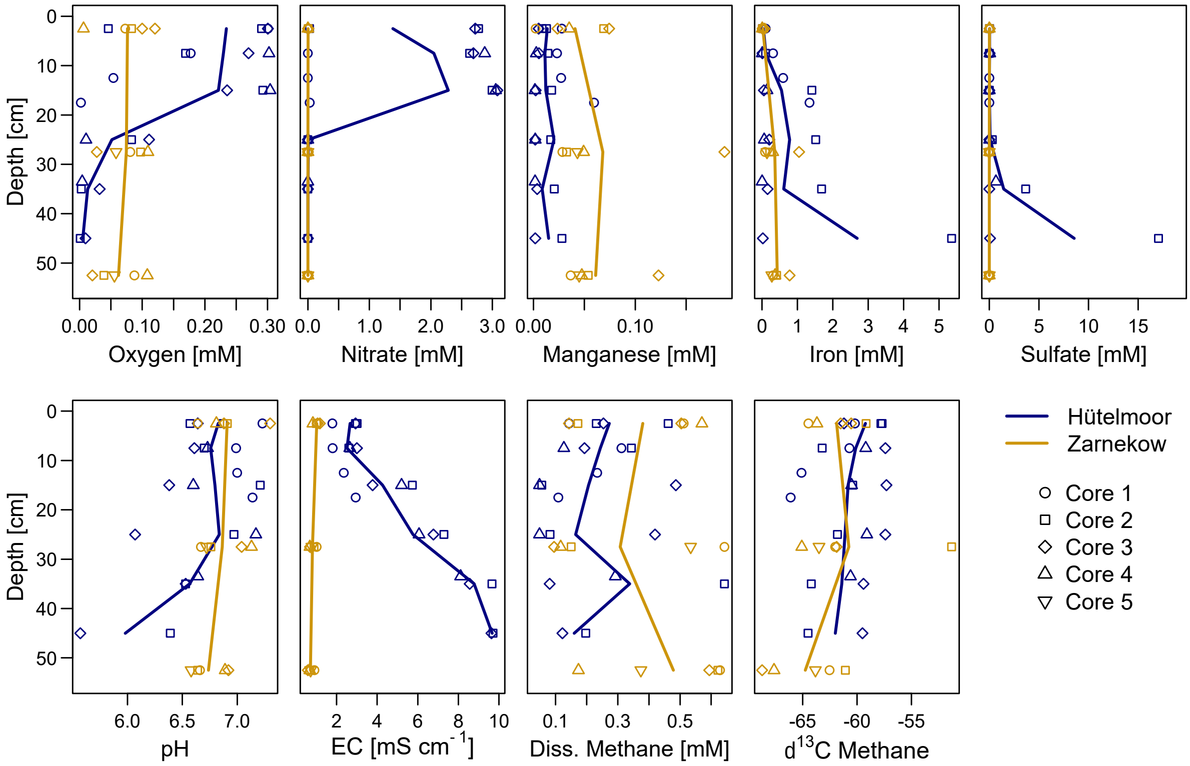
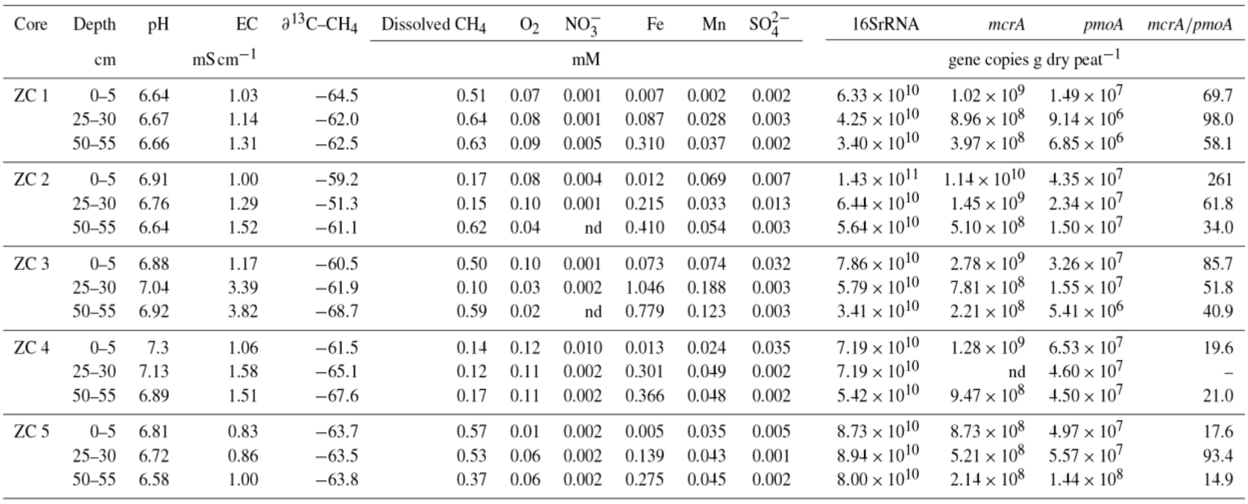


Figure A-2 – Depth profiles of oxygen, nitrate, total iron, manganese, and sulfate (a), and profiles of pH, EC, dissolved methane, and the isotopic signature of methane-bound carbon (b) in both study sites. Solid lines connect the respective means of individual wetlands. (n = 4 for Hütelmoor and n = 5 for Zarnekow).

Table A-1– Environmental conditions and geochemical conditions, and microbial abundances in peat cores from the Hütelmoor, a coastal minerotrophic fen in northeastern Germany. Environmental conditions are described by pH and EC (electrical conductivity). Geochemical parameters shown are dissolved methane (CH_4) concentrations, the isotopic signature of methane-bound carbon ($\partial^{13}C$–CH_4), and concentrations of terminal electron acceptors which are denoted with their respective chemical abbreviations. Microbial abundances here represent the mean value of subsamples for each depth section (n=2). nd: not detected.

Core	Depth	pH	EC	$\partial^{13}C$–CH_4	Dissolved CH_4	O_2	NO_3^-	Fe	Mn	SO_4^{2-}	16SrRNA	*mcrA*	*pmoA*	*mcrA/pmoA*
	cm		$mS\,cm^{-1}$		mM						gene copies g dry peat^{-1}			
HC 1	0–5	7.2	1.79	−60.2	0.14	0.30	nd	0.10	0.03	0.03	2.04×10^{10}	1.15×10^{8}	6.60×10^{6}	17.7
	5–10	7.0	1.80	−60.7	0.31	0.18	nd	0.31	0.02	0.01	3.25×10^{10}	3.36×10^{7}	6.68×10^{7}	0.51
	10–15	7.0	2.35	−65.1	0.23	0.05	nd	0.60	0.03	nd	2.11×10^{10}	8.12×10^{7}	1.76×10^{7}	6.12
	15–20	7.1	2.94	−66.1	0.11	nd	0.03	1.34	0.06	nd	3.08×10^{10}	1.21×10^{8}	2.76×10^{7}	4.41
HC 2	0–5	6.9	3.01	−57.8	0.46	0.05	0.03	0.03	0.01	nd	1.10×10^{11}	1.13×10^{10}	1.03×10^{7}	1170
	5–10	6.7	2.60	−63.2	0.34	0.17	2.63	0.10	0.01	0.01	5.51×10^{10}	7.27×10^{7}	1.69×10^{7}	4.73
	10–20	7.2	5.73	−60.4	0.06	0.29	3.00	1.41	0.02	nd	3.13×10^{10}	4.47×10^{6}	7.32×10^{6}	0.74
	20–30	7.0	7.29	−61.8	0.08	0.08	nd	1.51	0.02	0.29	4.71×10^{9}	6.41×10^{5}	4.50×10^{5}	3.75
	30–40	6.5	9.66	−64.2	0.64	nd	nd	1.68	0.02	3.66	2.09×10^{9}	6.21×10^{5}	3.90×10^{4}	18.3
	40–50	6.4	9.71	−64.5	0.20	nd	nd	5.35	0.03	17.1	4.09×10^{9}	2.47×10^{6}	2.75×10^{5}	10.7
HC 3	0–5	6.6	2.93	−57.7	0.23	0.29	2.77	0.11	0.01	0.04	1.10×10^{11}	1.34×10^{9}	3.51×10^{8}	3.86
	5–10	6.6	3.00	−57.4	0.19	0.27	2.69	0.01	0.01	0.03	8.72×10^{10}	1.40×10^{9}	3.42×10^{7}	46.6
	10–20	6.4	3.77	−57.3	0.49	0.24	3.08	0.05	nd	nd	6.08×10^{10}	5.86×10^{8}	9.35×10^{6}	63.6
	20–30	6.1	6.77	−57.4	0.42	0.11	nd	0.20	nd	nd	4.26×10^{10}	3.48×10^{8}	1.92×10^{7}	18.2
	30–40	6.5	8.56	−59.4	0.08	0.03	nd	0.16	nd	nd	1.05×10^{10}	3.20×10^{6}	1.17×10^{6}	2.74
	40–50	5.6	9.36	−59.5	0.12	0.01	nd	0.02	nd	0.08	3.18×10^{9}	2.16×10^{6}	2.58×10^{5}	8.39
HC 4	0–5	6.6	2.93	−61.2	0.25	0.30	2.72	0.02	0.01	0.04	1.17×10^{11}	3.63×10^{9}	3.09×10^{8}	11.7
	5–10	6.7	2.65	−59.2	0.13	0.30	2.87	0.01	nd	0.05	4.87×10^{10}	1.09×10^{9}	7.51×10^{7}	14.5
	10–20	6.6	5.20	−60.5	0.05	0.30	3.05	0.14	nd	nd	4.85×10^{10}	8.71×10^{8}	2.15×10^{7}	40.8
	20–30	7.2	6.06	−59.1	0.05	0.01	nd	0.06	nd	0.02	9.78×10^{9}	5.82×10^{7}	7.91×10^{6}	7.36
	30–40	6.6	8.11	−60.6	0.29	nd	nd	0.09	nd	0.67	1.60×10^{9}	1.58×10^{6}	1.25×10^{6}	1.27

Table A-2 – Environmental conditions and geochemical conditions, and microbial abundances in peat cores from Zarnekow, a freshwater minerotrophic fen in northeastern Germany. Environmental conditions are described by pH and EC (electrical conductivity). Geochemical parameters shown are dissolved methane (CH_4) concentrations, the isotopic signature of methane-bound carbon ($\partial^{13}C–CH_4$), and concentrations of terminal electron acceptors which are denoted with their respective chemical abbreviations. Microbial abundances here represent the mean value of subsamples for each depth section (n=2). nd: not detected.



Core	Depth	pH	EC	$\partial^{13}C–CH_4$	Dissolved CH_4	O_2	NO_3^-	Fe	Mn	SO_4^{2-}	16SrRNA	*mcrA*	*pmoA*	*mcrA/pmoA*
	cm		$mS\,cm^{-1}$				mM					gene copies g dry peat^{-1}		
ZC 1	0–5	6.64	1.03	−64.5	0.51	0.07	0.001	0.007	0.002	0.002	6.33×10^{10}	1.02×10^{9}	1.49×10^{7}	69.7
	25–30	6.67	1.14	−62.0	0.64	0.08	0.001	0.087	0.028	0.003	4.25×10^{10}	8.96×10^{8}	9.14×10^{6}	98.0
	50–55	6.66	1.31	−62.5	0.63	0.09	0.005	0.310	0.037	0.002	3.40×10^{10}	3.97×10^{8}	6.85×10^{6}	58.1
ZC 2	0–5	6.91	1.00	−59.2	0.17	0.08	0.004	0.012	0.069	0.007	1.43×10^{11}	1.14×10^{10}	4.35×10^{7}	261
	25–30	6.76	1.29	−51.3	0.15	0.10	0.001	0.215	0.033	0.013	6.44×10^{10}	1.45×10^{9}	2.34×10^{7}	61.8
	50–55	6.64	1.52	−61.1	0.62	0.04	nd	0.410	0.054	0.003	5.64×10^{10}	5.10×10^{8}	1.50×10^{7}	34.0
ZC 3	0–5	6.88	1.17	−60.5	0.50	0.10	0.001	0.073	0.074	0.032	7.86×10^{10}	2.78×10^{9}	3.26×10^{7}	85.7
	25–30	7.04	3.39	−61.9	0.10	0.03	0.002	1.046	0.188	0.003	5.79×10^{10}	7.81×10^{8}	1.55×10^{7}	51.8
	50–55	6.92	3.82	−68.7	0.59	0.02	nd	0.779	0.123	0.003	3.41×10^{10}	2.21×10^{8}	5.41×10^{6}	40.9
ZC 4	0–5	7.3	1.06	−61.5	0.14	0.12	0.010	0.013	0.024	0.035	7.19×10^{10}	1.28×10^{9}	6.53×10^{7}	19.6
	25–30	7.13	1.58	−65.1	0.12	0.11	0.002	0.301	0.049	0.002	7.19×10^{10}	nd	4.60×10^{7}	–
	50–55	6.89	1.51	−67.6	0.17	0.11	0.002	0.366	0.048	0.002	5.42×10^{10}	9.47×10^{8}	4.50×10^{7}	21.0
ZC 5	0–5	6.81	0.83	−63.7	0.57	0.01	0.002	0.005	0.035	0.005	8.73×10^{10}	8.73×10^{8}	4.97×10^{7}	17.6
	25–30	6.72	0.86	−63.5	0.53	0.06	0.002	0.139	0.043	0.001	8.94×10^{10}	5.21×10^{8}	5.57×10^{7}	93.4
	50–55	6.58	1.00	−63.8	0.37	0.06	0.002	0.275	0.045	0.002	8.00×10^{10}	2.14×10^{8}	1.44×10^{8}	14.9

Community composition of bacteria and archaea

Bacterial sequences could be affiliated into a total of 30 bacterial phyla (Fig. A-3). Among them, Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Nitrospirae, and Bacteroidetes were present in all samples. With mean relative abundance of 48 %, Proteobacteria was the most abundant phylum. Some taxa (e.g., Verrucomicrobia; Atribacteria, OP9; and AD3) were present only in Hütelmoor. Variation in community composition was larger in Hütelmoor samples than in Zarnekow.

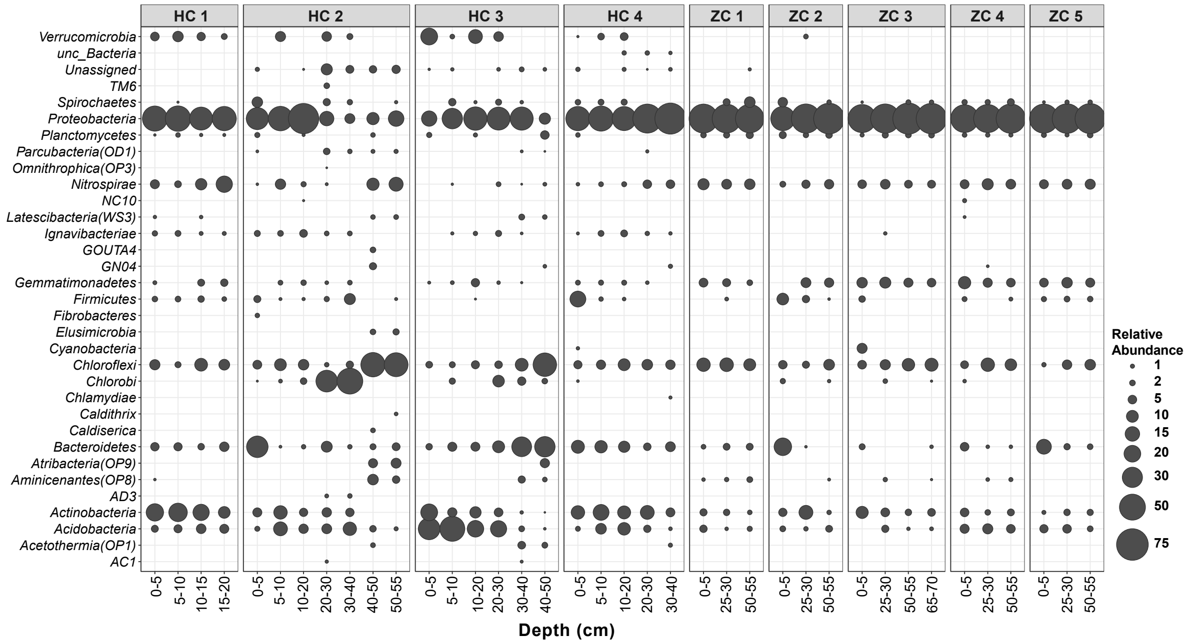


Figure A-3 – Relative abundances of different bacterial lineages in the study sites. Along the horizontal axis samples are arranged according to site and depth. The rank order along the vertical axis is shown for the phylum level.

Within Proteobacteria, the alpha subdivision was the most dominant group, having contributed 26.7 % to all the libraries on average (Fig. A-4). The family Hyphomicrobiaceae dominated the Alphaproteobacteria and was distributed evenly across samples but missing in the surface and bottom peat layers in HC 2. In addition, methanotrophs were clearly in low abundance across all samples, representing only 0.06 % and 0.05 % of the bacterial community in Hütelmoor and Zarnekow, respectively. Of the few methanotrophs that were detected, type II methanotrophs (mainly Methylocystaceae) outcompeted type I methanotrophs (mainly Methylococcaceae) in the community, while members of the genus *Methylocella* were absent (Fig. A-4).

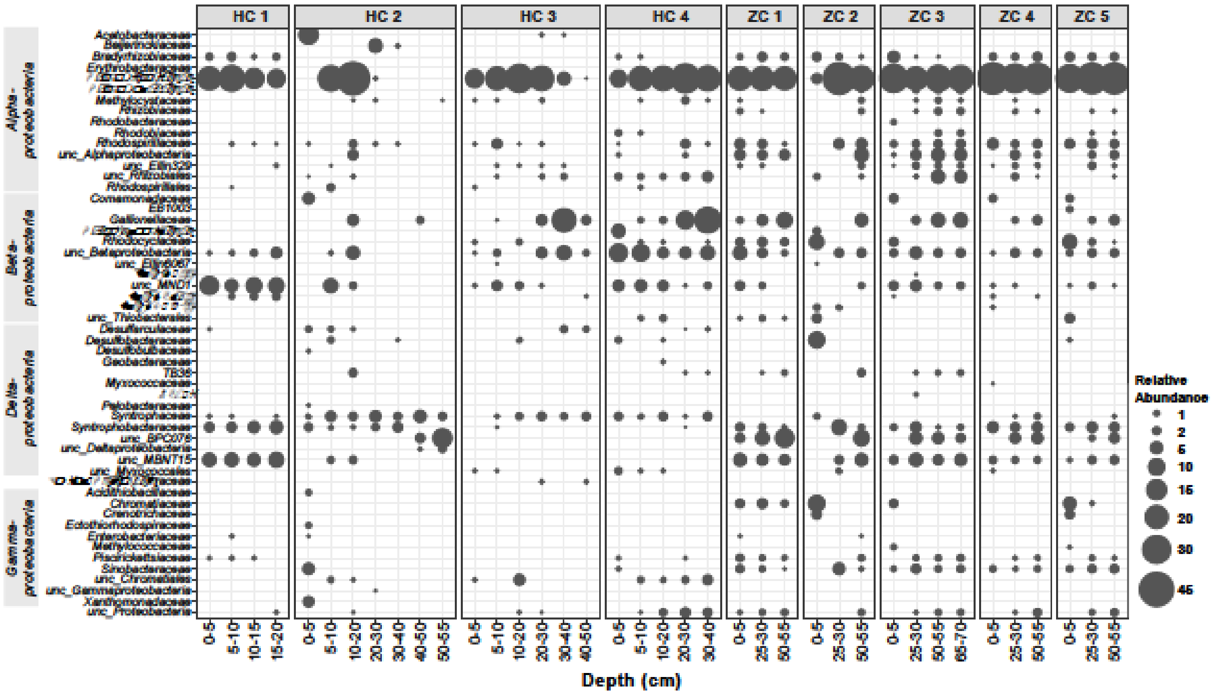


Figure A-4 – Relative abundances within Proteobacteria phylum in the study sites. Along the horizontal axis samples are arranged according to site and depth. The rank order along the vertical axis is shown for the family level. If an assignment to the family level was not possible, the next higher taxonomic level was used.

Within the archaeal community, Bathyarchaeota were mostly dominating over Euryarchaeota (Fig. A-5). The miscellaneous Crenarchaeota group (MCG; mainly the order of pGrfC26) in Bathyarchaeota prevailed across all samples but was especially abundant in HC 2 samples. In addition to Bathyarchaeota, methanogenic archaea were important and on average contributed 30.6 % to the whole archaeal community. Among the methanogens, acetoclastic methanogens were more abundant in most of the samples and Methanosaetaceae (24.8 %) were the major component. They were present in most samples and much more dominant than Methanosarcinaceae (2.0 %). Hydrogenotrophic methanogens, such as Methanomassiliicoccaceae (1.6 %), Methanoregulaceae (1.2 %), and Methanocellaceae (0.6 %), albeit low in abundance, were detected in many samples. Hütelmoor samples displayed greater variability in archaeal community composition compared to Zarnekow samples. The putative anaerobic methanotrophs of the ANME-2d (Raghoebarsing et al., 2006) clade occurred in patchy abundance with dominance in single spots of both sites. In HC 1 they represented a mean relative abundance of 40.9 % of total archaeal reads but were almost absent in all other Hütelmoor cores. In Zarnekow core 3, ANME-2d represented up to approximately 30 % of all archaea but were otherwise low in abundance.

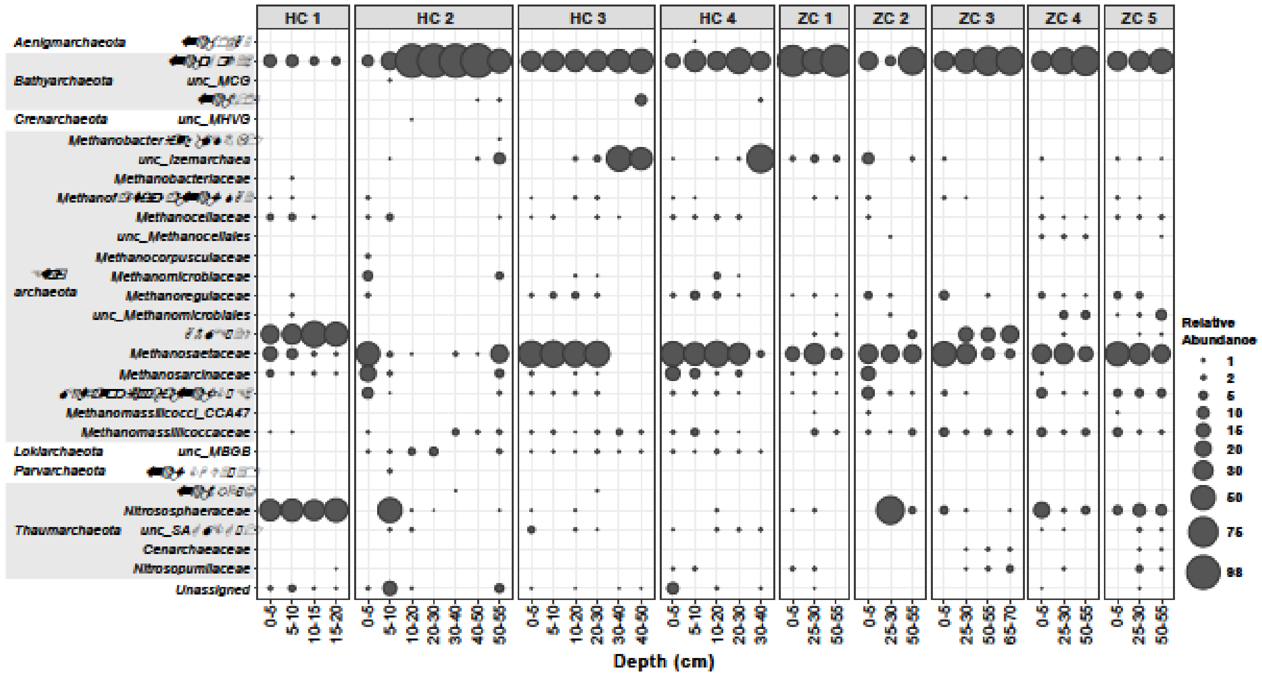


Figure A-5 – Relative abundances of different archaeal lineages in the study sites. Along the horizontal axis samples are arranged according to site and depth. The rank order along the vertical axis is shown for the family level. If an assignment to the family level was not possible, the next higher taxonomic level was used.

Environmental drivers of microbial community composition

Bacterial and archaeal population at both peatland sites showed distinct clustering (Fig. A-6) with similarly high intra- and inter-site variations but greater overall variation in community composition in the Hütelmoor. Community composition varied much more strongly in HC 2 than in any other core (Fig. A-6). Bacterial communities in HC 1 were more similar to communities in all Zarnekow cores than in other Hütelmoor cores (Fig. A-6a). The archaeal community in HC 1 was more similar to Zarnekow cores as well (Fig. A-6b). Environmental fit vectors suggest pH, oxygen, and alternative TEA availability as important factors influencing microbial community composition. The EC vector suggests the importance of brackish conditions in shaping microbial communities in the Hütelmoor (Fig. A-6a–c).

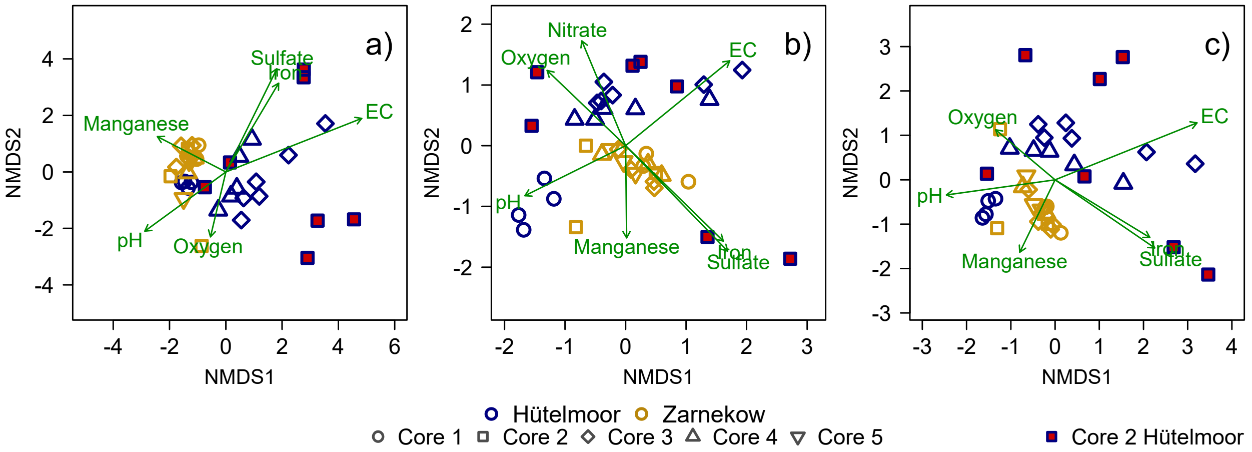


Figure A-6 – NMDS plots showing (a) bacterial, (b) archaeal, and (c) microbial (bacterial plus archaeal) community composition across the nine peat cores. The point positions represent distinct microbial communities, with the border colors of the symbols referring to the study sites and their shapes representing the core number. HC 2 symbols are highlighted with red fill to emphasize the large variation in microbial community within the core. Environmental fit vectors with a significance of p<0.05 are shown in green.

Total microbial and functional gene abundances

Quantitative PCR results show that, in both fens, *mcrA* abundance is up to 2 orders of magnitude greater than *pmoA* abundance (Fig. A-7, Tables A-1 and A-2). Gene copy numbers of *mcrA* are overall higher and spatially more stable in Zarnekow than in Hütelmoor. Total microbial abundance declined with depth more strongly in Hütelmoor than in Zarnekow (Fig. A-7). There was a pronounced decrease in microbial abundances at 20 cm depth in the Hütelmoor. For example, 16S rRNA gene and *pmoA* gene copy numbers in deeper samples (below 20 cm depth) are 1 order of magnitude lower than in upper samples on average, while the *mcrA* gene abundances are approximately 2 orders of magnitude lower. Hütelmoor samples also exhibited larger heterogeneity in terms of abundances than Zarnekow samples. Contrary to previous studies, methanotroph abundance did not correlate with dissolved CH_4 or oxygen concentrations.

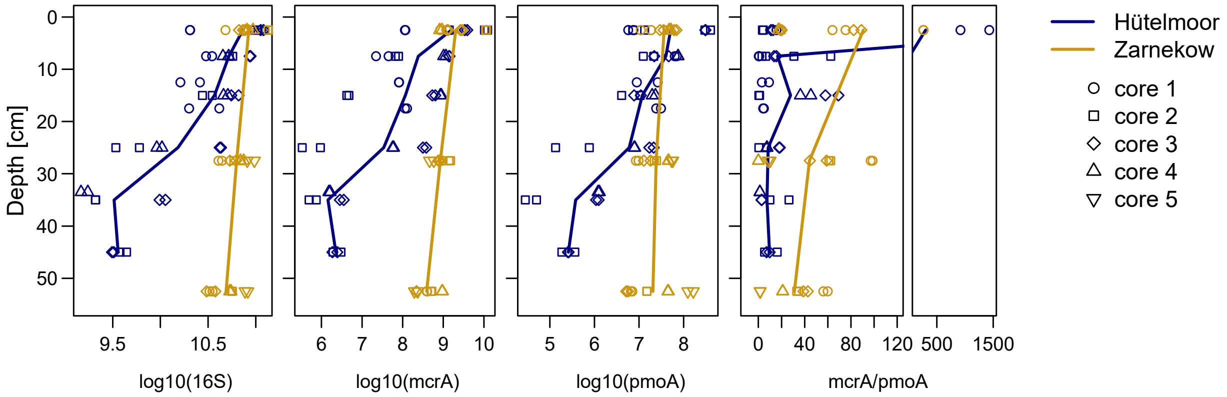


Figure A-7 – Depth distribution of qPCR abundances for total microbial (16S), methanogen (mcrA), methanotroph (pmoA), and ratio of mcrA to pmoA gene copy numbers in both sites. Microbial abundances were designated as numbers of gene copies per gram of dry peat soil. Duplicate measurements per depth section are shown against sampling depth using log-transformed values. Solid lines indicate mean abundances for individual wetlands (n=4 for Hütelmoor and n=5 for Zarnekow). Note that the plot at the right was split into two plots to capture very high mcrA/pmoA *ratios in the upper peat layer.*

Discussion

Fen geochemistry and relations to microbial community composition

The rewetting of drained fens promotes elevated CH_4 production and emission, which can potentially offset carbon sink benefits. Few studies have attempted to link microbial community dynamics and site geochemistry with observed patterns in CH_4 production and/or emission in rewetted fens, while such data are crucial for predicting long-term changes to CH_4 cycling (Galand et al., 2002; Yrjälä et al., 2011; Juottonen et al., 2012). In this study, we show that CH_4-cycling microbial community composition is related to patterns in site geochemistry in two rewetted fens with high CH_4 emissions, high methanogen abundances, and low methanotroph abundances. Our results suggest that high methanogen abundances concurrent with low methanotroph abundances are characteristic of rewetted fens with ongoing high CH_4 emissions. Thus, we present microbial evidence for sustained elevated CH_4 emissions in mostly inundated rewetted temperate fens.

The environmental conditions and associated geochemistry of the two rewetted fens were largely different. Depth profiles of porewater geochemical parameters show the fens differed in EC throughout the entire peat profile, while pH and concentrations of alternative TEAs differed at certain depths. In general, concentrations of TEAs oxygen, sulfate, nitrate, and iron were higher in the Hütelmoor. In Zarnekow, geochemical conditions varied little across the fen and along the peat depth profiles (Fig. A-2). As expected, the geochemical heterogeneity was reflected in microbial community structure in both sites, suggesting the importance of environmental characteristics and associated geochemical conditions as drivers of microbial community composition (Figs. A-2, A-3, A-4, A-6). The NMDS ordinations (Fig. A-6) show large variation in archaeal and bacterial community composition in the coastal brackish fen and much less variation in the freshwater riparian fen. Environmental fit vectors (Fig. A-6) suggest that salinity (indicated by the EC vector), pH, oxygen, and alternative TEA availability are the most important measured factors influencing microbial communities in the two fens. Patterns in microbial community composition have previously been linked to salinity (e.g., Chambers et al., 2016; Wen et al., 2017), pH (e.g., Yrjälä et al., 2011; Wen et al., 2017), and TEA availability in peatlands (e.g., He et al., 2015).

Comparing the geochemical depth profiles (Fig. A-2) with the relative abundance of bacteria and archaea (Figs. A-3 and A-4) provides a more complete picture of the relationships between microbial communities and site geochemistry, particularly with respect to TEA utilization. While the porewater depth profiles suggest there is little nitrate available for microbial use in HC 1, the relative abundance plot for Archaea showed that this core was dominated by ANME-2d. ANME-2d were recently discovered to be anaerobic methanotrophs that oxidize CH_4, performing reverse methanogenesis using nitrate as an electron acceptor (Haroon et al., 2013). However, ANME-2d has also been implicated in the iron-mediated anaerobic oxidation of methane (Ettwig et al., 2016), and the HC 1 site showed slightly higher total iron concentrations. The relevance of ANME-2d as CH_4 oxidizers in terrestrial habitats is still not clear (Winkel et al., 2018). Rewetting converts the fens into widely anaerobic conditions, thus providing conditions suitable for the establishment of anaerobic oxidation of methane, but this has yet to be demonstrated in fens. The patchy yet locally high abundance of ANME-2d both in Hütelmoor and in Zarnekow suggests an ecological relevance of this group. Shifts towards less negative $\delta^{13}C\text{-}CH_4$ signatures in the upper peat profile, for example, from −65 ‰ to −60 ‰ in HC 1 (where ANME-2d was abundant), may indicate that partial oxidation of CH_4 occurred, but we could only speculate whether or not ANME-2d are actively involved in this CH_4 oxidation.

Although TEA input may be higher in the Hütelmoor, here, methanogenic conditions also predominate. This finding contrasts the measured oxygen concentrations in the upper peat profile, as methanogenesis under persistently oxygenated conditions is thermodynamically not possible. However, seasonal analysis of oxygen concentrations in both sites suggests highly fluctuating oxygen regimes both spatially and temporary (data not shown). Such non-uniform distribution of redox processes has already been described elsewhere, in particular for methanogenesis (Hoehler et al., 2001; Knorr et al., 2009). It is possible that oxygen levels in both fens are highly variable, allowing for both aerobic and anaerobic carbon turnover processes. Recent studies from wetlands show that methanogenesis can occur in aerobic layers, driven mainly by Methanosaeta (Narrowe et al., 2017; Wagner, 2017), which were detected in a high abundance in this study (Fig. A-5). Further, oxygen may not necessarily be available within aggregates entailing anaerobic pathways and, thus, the existence of anaerobic microenvironments may also partially explain the seemingly contradictory co-occurrence of oxygen and the highly abundant methanogens. Anaerobic conditions are also reflected by the extensive and stable occurrence of the strictly anaerobic syntrophs (e.g., Syntrophobacteraceae, Syntrophaceae) in most samples, even in the top centimeters. This suggests that syntrophic degradation of organic material is taking place in the uppermost layer and the fermented substances are readily available for methanogens. As geochemistry and microbial community composition differ among the sites in this study, it is thus notable that a similarly high abundance of methanogens, and low abundance of methanotrophs, was detected in both fens. The dominance of methanogens implies that readily available substrates and favorable geochemical conditions promote high anaerobic carbon turnover despite seasonally fluctuating oxygen concentrations in the upper peat layer.

Low methanotroph abundances in rewetted fens

Methanogens (mainly Methanosaetaceae) dominated nearly all of the various niches detected in this study, while methanotrophs were highly under-represented in both sites (Figs. A-3 and A-4). Functional and ribosomal gene copy numbers not only show a high ratio of methanogen to methanotroph abundance (Fig. A-7), irrespective of site and time of sampling, but also a small contribution of methanotrophs to total bacterial population in both sites. Methanotrophs constitute only ~ 0.06 % of the total bacterial population in the Hütelmoor and ~ 0.05 % at Zarnekow. It should be noted that in this study we measured only gene abundances and not transcript abundances, and the pool both of active methanogens and methanotrophs was likely smaller than the numbers presented here (Freitag and Prosser, 2009; Freitag et al., 2010; Cheema et al., 2015; Franchini et al., 2015). Also, as we were unable to obtain microbial samples from before rewetting, a direct comparison of microbial abundances was not possible. This was, therefore, not a study of rewetting effects. For this reason, we performed an exhaustive literature search on relevant studies of pristine fens. Compared to pristine fens, we detected a low abundance of methanotrophs. Liebner et al. (2015), for example, found methanotrophs represented 0.5 % of the total bacterial community in a pristine, subarctic transitional bog–fen palsa, while *mcrA* and *pmoA* abundances were nearly identical. In a pristine Swiss alpine fen, Liebner et al. (2012) found methanotrophs generally outnumbered methanogens by an order of magnitude. Cheema et al. (2015) and

Franchini et al. (2015) reported *mcrA* abundances higher than *pmoA* abundances by only 1 order of magnitude in a separate Swiss alpine fen. In the rewetted fens in our study, *mcrA* gene abundance was up to 2 orders of magnitude higher than *pmoA* abundance (Fig. A-7). Due to inevitable differences in methodology and equipment, direct comparisons of absolute gene abundances are limited. Therefore, only the abundances of methanotrophs relative to methanogens and relative to the total bacterial community were compared, rather than absolute abundances. We are confident that this kind of "normalization" can mitigate the bias of different experiments and allows a comparison of sites. Further, all primers and equipment used in this study were identical to those used by Liebner et al. (2012, 2015), making the comparison more reliable.

As most methanotrophs live along the oxic–anoxic boundary of the peat surface and plant roots therein (Le Mer and Roger 2001), the low methanotroph abundances in both fens could be explained by disturbances to this boundary zone and associated geochemical pathways following inundation. In rewetted fens, a massive plant dieback has been observed along with strong changes in surface peat geochemistry (Hahn-Schöfl et al., 2011; Hahn et al., 2015). In addition to substrate (i.e., CH_4) availability, oxygen availability is the most important factor governing the activity of most methanotrophs (Le Mer and Roger 2001; Hernandez et al., 2015). The anoxic conditions at the peat surface caused by inundation may have disturbed existing methanotrophic niches – either directly by habitat destruction and/or indirectly by promoting the growth of organisms that are able to outcompete methanotrophs for oxygen. Heterotrophic organisms, for example, have been shown to outcompete methanotrophs for oxygen when oxygen concentrations are greater than 5 µM (van Bodegom et al., 2001). Our microbial data support this conclusion, as Hyphomicrobiaceae, most of which are aerobic heterotrophs, was the most abundant bacterial family in both fens. Incubation data from Zarnekow (Fig. A-S1 in the Supplement) show that the CH_4 oxidation potential is high; however, incubations provide ideal conditions for methanotrophs and thus only potential rates. It is likely that, *in situ*, the activity of methanotrophs is overprinted by the activity of competitive organisms such as heterotrophs. It is also possible that methane oxidation may occur in the water column above the peat surface, but this was beyond the scope of this study. Nevertheless, oxidation rates are low enough that emissions remain high, as demonstrated by the high dissolved CH_4 concentrations and ongoing high fluxes.

Comparable studies have so far been conducted in nutrient-poor or mesotrophic fens where post-rewetting CH_4 emissions, though higher than pre-rewetting, did not exceed those of similar pristine sites (e.g., Yrjälä et al., 2011; Juottonen et al., 2005, 2012). Nevertheless, there is mounting evidence linking CH_4-cycling microbe abundances to CH_4 dynamics in rewetted fens. Juottonen et al. (2012), for example, compared *pmoA* gene abundances in three natural and three rewetted fens and found them to be lower in rewetted sites. The same study also measured a lower abundance of *mcrA* genes in rewetted sites, which was attributed to a lack of available labile organic carbon compounds. In peatlands, and especially fens, litter and root exudates from vascular plants can stimulate CH_4 emissions (Megonigal et al., 2005; Bridgham et al., 2013; Agethen and Knorr 2018), and excess labile substrate has been proposed as one reason for substantial increases in CH_4 emissions in rewetted fens (Hahn-Schöfl et al., 2011). Future studies should compare pre- and post-rewetting microbial abundances along with changes in CH_4 emissions, plant communities, and peat geochemistry to better assess the effect rewetting has on the CH_4-cycling microbial community.

Conclusions

Despite a recent increase in the number of rewetting projects in northern Europe, few studies have characterized CH_4-cycling microbes in restored peatlands, especially fens. In this study, we show that rewetted fens differing in geochemical conditions and microbial community composition have a similarly low abundance of methanotrophs, a high abundance of methanogens, and an established anaerobic carbon cycling microbial community. Comparing these data to pristine wetlands with lower CH_4 emission rates, we found that pristine wetlands have a higher abundance of methanotrophs than measured in the fens in this study, suggesting the inundation and associated anoxia caused by flooding may disturb methanotrophic niches and negatively affect the ability of methanotrophic communities to establish. The abundances of methane producers and consumers are thus suggested as indicators of continued elevated CH_4 emissions following the rewetting of drained fens. Management decisions regarding rewetting processes should consider that disturbances to methanotrophic niches are possible if rewetting leads to long-term inundation of the peat surface.

Acknowledgements

This study was conducted within the framework of the Research Training Group “Baltic TRANSCOAST” funded by the DFG (Deutsche Forschungsgemeinschaft) under grant number GRK 2000. The financial support to Xi Wen (grant no. 201408620031) provided by the China Scholarship Council (CSC), and to Matthias Winkel (ARCSS-1500931) provided by the National Science Foundation (NSF), is gratefully acknowledged. This study was supported by the Helmholtz Gemeinschaft (HGF) by funding the Helmholtz Young Investigators Group of Susanne Liebner (VH-NG-919) and Torsten Sachs (grant VH-NG-821), a Helmholtz Postdoc Programme grant to Franziska Koebsch (grant PD-129), and further supported by the Terrestrial Environmental Observatories (TERENO) Network. The Leibniz Institute for Baltic Sea Research (IOW) is also acknowledged for funding the lab work in this study. The European Social Fund (ESF) and the Ministry of Education, Science and Culture of Mecklenburg-Western Pomerania funded this work within the scope of the project WETSCAPES (ESF/14-BM-A55-0030/16). Matthias Gehre, head of the Laboratory of Stable Isotopes at the Helmholtz Centre for Environmental Research, is acknowledged for providing carbon isotope measurements for this study. Anke Saborowski and Anne Köhler are also acknowledged for support in the laboratory.

References

Abdalla, M., Hastings, A., Truu, J., Espenberg, M., Mander, U., and Smith, P.: Emissions of methane from northern peatlands: a review of management impacts and implications for future management options, Ecol. Evol., 6, 7080–7102, https://doi.org/10.1002/ece3.2469, 2016.

Agethen, S. and Knorr, K.-H.: Juncus effusus mono-stands in restored cutover peat bogs – Analysis of litter quality, controls of anaerobic decomposition, and the risk of secondary carbon loss, Soil Biology and Biochemistry, 117, 139–152, https://doi.org/10.1016/j.soilbio.2017.11.020, 2018.

Augustin, J. and Chojnicki, B.: Austausch von klimarelevanten Spurengasen, Klimawirkung und Kohlenstoffdynamik in den ersten Jahren nach der Wiedervernässung von degradiertem Niedermoorgrünland (Exchange of climate relevant trace gases, climate effect and carbon dynamics in the first years after re-wetting of degraded fen grassland), in: Phosphor- und Kohlenstoff Dynamik und Vegetationsentwicklung in wiedervernässten Mooren des Peenetals in Mecklenburg-Vorpommern (Phosphorus and carbon dynamics and vegetation development in re-wetted peatland of the Peene valley in Mecklenburg-Western Pomerania), edited by: Gelbrecht, J., Zak, D., and Augustin, J., Leibniz-Institut für Gewässerökologie und Binnenfischerei, Berlin, 50– 67, 2008 (in German).

Augustin, J., Merbach, W., and Rogasik, J.: Factors influencing nitrous oxide and methane emissions from minerotrophic fens in northeast Germany, Biol. Fert. Soils, 28, 1–4, https://doi.org/10.1007/s003740050455, 1998.

Blodau, C.: Thermodynamic control on terminal electron transfer and methanogenesis, in: Aquatic Redox Chemistry, ACS Symposium Series, edited by: Tratnyek, P. G., Grundl, T. J., and Haderlein, S. B., American Chemical Society, Oxford University Press Inc., Washington, D.C., 65–82, https://doi.org/10.1021/bk-2011-1071.ch004, 2011.

Bolger, A. M., Lohse, M., and Usadel, B.: Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics, 30, 2114–2120, https://doi.org/10.1093/bioinformatics/btu170, 2014.

Bridgham, S. D., Cadillo-Quiroz, H., Keller, J. K., and Zhuang, Q.: Methane emissions from wetlands: biogeochemical, microbial, and modeling perspectives from local to global scales, Global Change Biol., 19, 1325–1356, https://doi.org/10.1111/gcb.12131, 2013.

Cadillo-Quiroz, H., Yashiro, E., Yavitt, J. B., and Zinder, S. H.: Characterization of the archaeal community in a minerotrophic fen and terminal restriction fragment length polymorphism-directed isolation of a novel hydrogenotrophic methanogen, Appl. Environ. Microb. 74, 2059–2068, https://doi.org/10.1128/AEM.02222-07, 2008.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R.: QIIME allows analysis of high-throughput community sequencing data, Nat. Methods, 7, 335–336, https://doi.org/10.1038/nmeth.f.303, 2010.

Chambers, L. G., Guevara, R., Boyer, J. N., Troxler, T. G., and Davis, S. E.: Effects of salinity and inundation on microbial community structure and function in a mangrove peat soil, Wetlands, 36, 361–371, https://doi.org/10.1007/s13157-016-0745-8, 2016.

Chasar, L. S., Chanton, J. P., Glaser, P. H., and Siegel, D. I.: Methane concentration and stable isotope distribution as evidence of rhizospheric processes: comparison of a fen and bog in the

Glacial Lake Agassiz Peatland complex, Ann. Bot.-London, 86, 655–663, https://doi.org/10.1006/anbo.2000.1172, 2000.

Cheema, S., Zeyer, J., and Henneburger, R.: Methanotrophic and methanogenic communities in Swiss alpine fens dominated by Carex rostrata and Eriphorum angustifolium, Appl. Environ. Microb., 81, 5832–5844, https://doi.org/10.1128/AEM.01519-15, 2015.

Conrad, R.: Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N2O, and NO), Microbiol. Rev., 60, 609–640, 1996.

Degelmann, D. M., Borken, W., Drake, H. L., and Kolb, S.: Different atmospheric methane-oxidizing communities in European beech and Norway spruce soils, Appl. Environ. Microb., 76, 3228–3235, https://doi.org/10.1128/AEM.02730-09, 2010.

Emsens, W.-J., Aggenbach, C. J. S., Schoutens, K., Smolders, A. J. P., Zak, D., and van Diggelen, R.: Soil iron content as a predictor of carbon and nutrient mobilization in rewetted fens, PLoS ONE, 11, e0153166, https://doi.org/10.1371/journal.pone.0153166, 2016.

Ettwig, K. F., Zhu, B., Speth, D., Keltjens, J. T., Jetten, M. S. M., and Kartal, B.: Archaea catalyze iron-dependent anaerobic oxidation of methane, P. Natl. Acad. Sci. USA, 113, 12792–12796, https://doi.org/10.1073/pnas.1609534113, 2016.

Franchini, A. G., Henneberger, R., Aeppli, M., and Zeyer, J.: Methane dynamics in an alpine fen: a field-based study on methanogenic and methanotrophic microbial communities, FEMS Microbiology Ecology, 91, 1–13, https://doi.org/10.1093/femsec/fiu032, 2015.

Franz, D., Koebsch, F., Larmanou, E., Augustin, J., and Sachs, T.: High net CO_2 and CH_4 release at a eutrophic shallow lake on a formerly drained fen, Biogeosciences, 13, 3051–3070, https://doi.org/10.5194/bg-13-3051-2016, 2016.

Freitag, T. E. and Prosser, J. I.: Correlation of methane production and functional gene transcription activity in a peat soil, Appl. Environ. Microb., 75, 6679–6687, https://doi.org/10.1128/AEM.01021-09, 2009.

Freitag, T. E., Toet, S., Ineson, P., and Prosser, J. I.: Links between methane flux and transcriptional activities of methanogens and methane oxidizers in a blanket peat bog, FEMS Microbiology Ecology, 73, 157–165, https://doi.org/10.1111/j.1574-6941.2010.00871.x, 2010.

Galand, P. E., Saarnio, S., Fritze, H., and Yrjälä, K.: Depth related diversity of methanogen Archaea in Finnish oligotrophic fen, FEMS Microbiology Ecology, 42, 441–449, https://doi.org/10.1111/j.1574-6941.2002.tb01033.x, 2002.

Galand, P. E., Fritze, H., Conrad, R., and Yrjälä, K.: Pathways for methanogenesis and diversity of methanogenic archaea in three boreal peatland ecosystems, Appl. Environ. Microb., 71, 2195–2198, https://doi.org/10.1128/AEM.71.4.2195-2198.2005, 2005.

Hahn, J., Köhler, S., Glatzel, S., and Jurasinski, G.: Methane exchange in a coastal fen the first year after flooding – a systems shift, PLoS ONE, 10, e0140657, https://doi.org/10.1371/journal.pone.0140657, 2015.

Hahn-Schöfl, M., Zak, D., Minke, M., Gelbrecht, J., Augustin, J., and Freibauer, A.: Organic sediment formed during inundation of a degraded fen grassland emits large fluxes of CH_4 and CO_2, Biogeosciences, 8, 1539–1550, https://doi.org/10.5194/bg-8-1539-2011, 2011.

Haroon, M. F., Hu, S., Shi, Y., Imelfort, M., Keller, J., Hugenholtz, P., Yuan, Z., and Tyson, G.: Anaerobic oxidation of methane couple to nitrate reduction in a novel archaeal lineage, Nature, 500, 567–570, https://doi.org/10.1038/nature12375, 2013.

He, S., Malfatti, S. A., McFarland, J. W., Anderson, F. E., Pati, A., Huntemann, M., Tremblay, J., del Rio, T. G., Waldrop, M. P., Windham-Myers, L., and Tringe, S. G.: Patterns in wetland microbial community composition and functional gene repertoire associated with methane emissions, mBio, 6, 1–15, https://doi.org/10.1128/mBio.00066-15, 2015.

Herlemann, D. P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J., and Andersson, A. F.: Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea, ISME J., 5, 1571–1579, https://doi.org/10.1038/ismej.2011.41, 2011.

Hernandez, M. E., Beck, D. A., Lidstrom, M. E., and Chistoserdova, L.: Oxygen availability is a major factor in determining the composition of microbial communities involved in methane oxidation, PeerJ, 3, e801, https://doi.org/10.7717/peerj.801, 2015.

Hoehler, T. M., Alperin, M. J., Albert, D. B., and Martens, C. S.: Apparent minimum free energy requirements for methanogenic Archaea and sulfate-reducing bacteria in an anoxic marine sediment, FEMS Microbiology Ecology, 38, 33–41, https://doi.org/10.1016/S0168-6496(01)00175-1, 2001.

Jerman, V., Metje, M., Mandic-Mulec, I., and Frenzel, P.: Wetland restoration and methanogenesis: the activity of microbial populations and competition for substrates at different temperatures, Biogeosciences, 6, 1127–1138, https://doi.org/10.5194/bg-6-1127-2009, 2009.

Joosten, H., Brust, K., Couwenberg, J., Gerner, A., Holsten, B., Permien, T., Schäfer, A., Tanneberger, F., Trepel, M., and Wahren, A.: MoorFutures® Integration of additional ecosystem services (including biodiversity) into carbon credits – standard, methodology and transferability to other regions, Bundesamt für Naturschutz (Federal Ministry for the Environment, BfN), BfN Skripten 407, Bonn, Germany, 2015.

Juottonen, H., Galand, P. E., Tuittila, E.-S., Laine, J., Fritze, H., and Yrjälä, K.: Methanogen communities and bacteria along an ecohydrological gradient in a northern raised bog complex, Environ. Microbiol., 7, 1547–1557, https://doi.org/10.3389/fmicb.2015.00356, 2005.

Juottonen, H., Hynninen, A., Nieminen, M., Tuomivirta, T. T., Tuittila, E.-S., Nousiainen, H., Kell, D. K., Yrjälä, K., Tervahauta, A., and Fritze, H.: Methane-cycling microbial communities and methane emission in natural and restored peatlands, Appl. Environ. Microb., 78, 6386–6389, https://doi.org/10.1128/AEM.00261-12, 2012.

Jurasinski, G., Glatzel, S., Hahn, J., Koch, S., Koch, M., and Koebsch, F.: Turn on, fade out – Methane exchange in a coastal fen over a period of six years after rewetting, Geophys. Res. Abstr., 18, EGU2016-14899, 2016.

Keller, J. K. and Bridgham, S. D.: Pathways of anaerobic carbon cycling across an ombrotrophic-minerotrophic peatland gradient, Limnol. Oceanogr., 52, 96–107, https://doi.org/10.4319/lo.2007.52.1.0096, 2007.

Kelly, C. A., Dice, N. B., and Martens, C. S.: Temporal variations in the stable carbon isotopic composition of methane emitted from Minnesota peatlands, Global Biogeochem. Cy., 6, 263–269, https://doi.org/10.1029/92GB01478, 1992.

Knorr, K.-H. and Blodau, C.: Impact of experimental drought and rewetting on redox transformations and methanogenesis in mesocosms of a northern fen soil, Soil Biology and Biochemistry, 41, 1187–1198, https://doi.org/10.1016/j.soilbio.2009.02.030, 2009.

Knorr, K.-H., Lischeid, G., and Blodau, C.: Dynamics of redox processes in a minerotrophic fen exposed to a water table manipulation, Geoderma, 153, 379–392, https://doi.org/10.1016/j.geoderma.2009.08.023, 2009.

Koch, M., Koebsch, F., Hahn, J., and Jurasinski, G.: From meadow to shallow lake: Monitoring secondary succession in a coastal fen after rewetting by flooding based on aerial imagery and plot data, Mires Peat, 19, 1–17, https://doi.org/10.19189/MaP.2015.OMB.188, 2017.

Koebsch, F., Glatzel, S., and Jurasinski, G.: Vegetation controls emissions in a coastal brackish fen, Wetl. Ecol. Manag., 21, 323–337, https://doi.org/10.1007/s11273-013-9304-8, 2013.

Koebsch, F., Jurasinski, G., Koch, M., Hofmann, J., and Glatzel, S.: Controls for multi-scale temporal variation in ecosystem methane exchange during the growing season of a permanently inundated fen, Agr. Forest Meteorol., 204, 94–105, https://doi.org/10.1016/j.agrformet.2015.02.002, 2015.

Kolb, S., Knief, C., Stubner, S., and Conrad, R.: Quantitative detection of methanotrophs in soil by novel pmoA-targeted real-time PCR assays, Appl. Environ. Microb., 69, 2423–2429, https://doi.org/10.1128/AEM.69.5.2423-2429.2003, 2003.

Komulainen, V.-M., Nykanen, H., Martikainen, P. J., and Laine, J.: Short-term effect of restoration on vegetation change and methane emissions from peatlands drained for forestry in southern Finland, Can. J. Forest Res., 28, 402–411, https://doi.org/10.1139/x98-011, 1998.

Kowalski, N., Dellwig, O., Beck, M., Grunwald, M., Dürselen, C.-D., Badewien, T. H., Brumsack, H-J., van Beusekom, J. E. E., and Böttcher, M. E.: A comparative study of manganese dynamics in the water column and sediments of intertidal systems of the North Sea, Estuarine, Coast. Shelf Sci., 100, 3–17, https://doi.org/10.1016/j.ecss.2011.03.011, 2012.

Le Mer, J. and Roger. P.: Production, oxidation, emission, and consumption of methane by soils: a review, Eur. J. Soil Biol., 37, 25–50, https://doi.org/10.1016/S1164-5563(01)01067-6, 2001.

Liebner, S., Schwarzenbach, S. P., and Zeyer, J.: Methane emissions from an alpine fen in central Switzerland, Biogeochemistry, 109, 287–299, https://doi.org/10.1007/s10533-011-9629-4, 2012.

Liebner, S., Ganzert, L., Kiss, A., Yang, S., Wagner, D., and Svenning, M. M.: Shifts in methanogenic community composition and methane fluxes along the degradation of discontinuous permafrost, Front. Microbiol., 6, 1–10, https://doi.org/10.3389/fmicb.2015.00356, 2015.

Liu, D. Y., Ding, W. X., Jia, Z. J., and Cai, Z. C.: Relation between methanogenic archaea and methane production potential in selected natural wetland ecosystems across China, Biogeosciences, 8, 329–338, https://doi.org/10.5194/bg-8-329-2011, 2011.

Martin, M.: Cutadapt removes adapter sequences from high-throughput sequencing reads, EMBnet. Journal, 17, 10–12, https://doi.org/10.14806/ej.17.1.200, 2011.

McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., Andersen, G. L., Knight, R., and Hugenholtz, P.: An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea, ISME J,, 6, 610–618, https://doi.org/10.1038/2Fismej.2011.139, 2012.

Megonigal, J. P., Mines, M. E., and Visscher, P. T.: Anaerobic metabolism: linkages to trace gases and aerobic processes, in: Biogeochemistry, edited by: Schlesinger, W. H., Elsevier, Oxford, UK, 350–362, 2005.

Minke, M., Augustin, J., Burlo, A., Yarmashuk, T., Chuvashova, H., Thiele, A., Freibauer, A., Tikhonov, V., and Hoffmann, M.: Water level, vegetation composition, and plant productivity explain greenhouse gas fluxes in temperate cutover fens after inundation, Biogeosciences, 13, 3945–3970, https://doi.org/10.5194/bg-13-3945-2016, 2016.

Myhre, G., Shindell, D., Breon, F.-M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J.-F., Lee, D., Mendoza, B., Nakajima, T., Robock, A., Rotstayn, L., Stephens, G., and Zhang, H.: Anthropogenic and natural radiative forcing. Chapter 8, in: Climate Change 2013. The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovermental Panel on Climate Change, edited by: Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, D., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P. M., Cambridge University Press, Cambridge, UK and New York, USA, 659–740, 2013.

Narrowe, A. B., Angle, J. C., Daly, R. A., Stefanik, K. C., Wrighton, K. C., and Miller, C. S.: High-resolution sequencing reveals unexplored archaeal diversity in freshwater wetland soils, Environ. Microbiol., 19, 2192–2209, https://doi.org/10.1111/1462-2920.13703, 2017.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, Stevens, M. H. H., Szoecs, E., and Wagner, H.: vegan: Community Ecology Package, R package version 2.4-5, available at: https://CRAN.R-project.org/package=vegan (last access: 1 October 2018), 2017.

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

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F. O.: The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, Nucleic Acids Res., 41, D590–596, https://doi.org/10.1093/nar/gks1219, 2013.

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

R Core Team: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, available at: https://www.R-project.org/ (last access: 1 October 2018), 2017.

Schemel, L.: Simplified conversions between specific conductance and salinity units for use with data from monitoring stations, Interagency Ecological Program Newsletter, 14, 17–18, 2001.

Schönfeld-Bockholt, R., Roth, D., and Dittmann, L.: Ch. Teilflächenbezogene ökologische und futterwirtschaftliche Beurteilung des Grünlandes im Naturschutzgebiet Heiligensee und Hütelmoor, in: Effiziente Nutzung von Grünland als Ressource für die Milch- und Fleischproduktion Mitteilungen der Arbeitsgemeinschaft Grünland und Futterbau, Zollikofen, Switzerland, 2008.

Segers, R.: Methane production and methane consumption: a review of processes underlying wetland methane fluxes, Biogeochemistry, 41, 23–51, https://doi.org/10.1023/A:1005929032764, 1998.

Steffenhagen, P., Zak, D., Schulz, K., Timmerman, T., and Zerbe, S.: Biomass and nutrient stock of submersed and floating macrophytes in shallow lakes formed by rewetting of degraded fens, Hydrobiologia, 692, 99–109, https://doi.org/10.1007/s10750-011-0833-y, 2012.

Steinberg, L. M. and Regan, J. M.: mcrA-targeted real-time quantitative PCR method to examine methanogen communities, Appl. Environ. Microb., 75, 4435–4442, https://doi.org/10.1128/AEM.02858-08, 2009.

Sun, C. L., Brauer, S. L., Cadillo-Quiroz, H., Zinder, S. H., and Yavitt, J. B.: Seasonal changes in methanogenesis and methanogenic community in three peatlands, New York State, Front. Microbiol., 3, 1–8, https://doi.org/10.3389/fmicb.2012.00081, 2012.

Takai, K. and Horikoshi, K.: Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes, Appl. Environ. Microb., 66, 5066–5072,2000.

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

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

Urbanová, Z., Bárta, J., and Picek, T.: Methane emissions and methanogenic archaea on pristine, drained and restored mountain peatlands, central Europe, Ecosystems, 16, 664–677, https://doi.org/10.1007/s10021-013-9637-4, 2013.

van Bodegom, P., Stams, F., Mollema, L., Boeke, S., and Leffelaar, P.: Methane oxidation and the competition for oxygen in the rice rhizosphere, Appl. Environ. Microb., 67, 3586–3597, https://doi.org/10.1128/AEM.67.8.3586-3597.2001, 2001.

Vanselow-Algan, M., Schmidt, S. R., Greven, M., Fiencke, C., Kutzbach, L., and Pfeiffer, E.-M.: High methane emissions dominated annual greenhouse gas balances 30 years after bog rewetting, Biogeosciences, 12, 4361–4371, https://doi.org/10.5194/bg-12-4361-2015, 2015.

Voigtländer, U., Schmidt, J., and Scheller, W.: Pflege-und Entwicklungsplan NSG Heiligensee und Hütelmoor, 1996.

Wagner, D.: Effect of varying soil water potentials on methanogenesis in aerated marshland soils, Sci. Rep.-UK, 7, 14706, https://doi.org/10.1038/s41598-017-14980-y, 2017.

Weisner, E. and Schernewski, G.: Adaptation to climate change: a combined coastal protection and re-alignment scheme in a Baltic tourism region, J. Coast. Res., 65, 1963–1968, https://doi.org/10.2112/SI65-332.1, 2013.

Wen, X., Yang, S., Horn, F., Winkel, M., Wagner, D., and Liebner, S.: Global biogeographic analysis of methanogenic archaea identifies community-shaping factors of natural environments, Frontiers in Microbiology, 8, 1–13, https://doi.org/10.3389/fmicb.2017.01339, 2017.

Wickham, H.: ggplot2: Elegant Graphics for Data Analysis, Springer, New York, 2009.

Wilhelm, E., Batino, R., and Wilcock, R. J.: Low-pressure solubility of gases in liquid water, Chem. Rev., 77, 219–262, https://doi.org/10.1021/cr60306a003, 1977.

Wilson, D., Blain, D., Couwenburg, J., Evans, C. D., Murdiyarso, D., Page, S. E., Renou-Wilson, F., Rieley, J. O., Sirin, A., Strack, M., and Tuittila, E.-S.: Greenhouse gas emission factors associated with rewetting of organic soils, Mires Peat, 17, 1–28, https://doi.org/10.19189/MaP.2016.OMB.222, 2016.

Winkel, M., Mitzscherling, J., Overduin, P. P., Horn, F., Winterfeld, M., Rijkers, R., Grigoriev, M. N., Knoblauch, C., Mangelsdorf, K., Wagner, D., and Liebner, S.: Anaerobic methanotrophic communities thrive in deep submarine permafrost, Sci. Rep.-UK, 8, 1–13, https://doi.org/10.1038/s41598-018-19505-9, 2018.

Yrjälä, K., Tuomivirta, T. T., Juottonen, H., Putkinen, A., Lappi, K., Tuittila, E.-S., Penttila, T., Minkkinen, K., Laines, J., Peltoniemi, K., and Fritze, H.: CH_4 production and oxidation processes in a boreal fen ecosystem after long-term water table drawdown, Global Change Biol., 17, 1311–1320, https://doi.org/10.1111/j.1365-2486.2010.02290.x, 2011.

Zak, D. and Gelbrecht, J.: The mobilisation of phosphorus, organic carbon, and ammonium in the initial stage of fen rewetting (a case study from NE Germany), Biogeochemistry, 85, 141–151, https://doi.org/10.1007/s10533-007-9122-2, 2007.

Zak, D., Reuter, H., Augustin, J., Shatwell, T., Barth, M., Gelbrecht, J., and McInnes, R. J.: Changes of the CO_2 and CH_4 production potential of rewetted fens in the perspective of temporal vegetation shifts, Biogeosciences, 12, 2455–2468, https://doi.org/10.5194/bg-12-2455-2015, 2015.

Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A.: PEAR: a fast and accurate Illumina Paired-End reAd merger, Bioinformatics, 30, 614–620, https://doi.org/10.1093/bioinformatics/btt593, 2014.

Zhou, X., Zhang, Z., Tian, L., Li, X., and Tian, C.: Microbial communities in peatlands along a chronosequence on the Sanjiang Plain, China, Nature Scientific Reports, 7, 1–11, https://doi.org/10.1038/s41598-017-10436-5, 2017.

Supplement

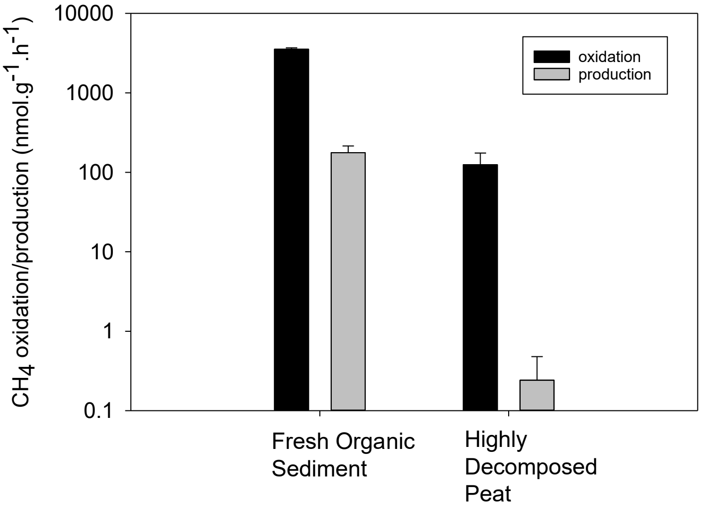


Figure A-S1 – Incubation data from Zarnekow, a freshwater minerotrophic fen in Northeastern Germany. Rates of methane production (n=3) and methane oxidation (n=3) are shown for both fresh (surficial) organic sediment and the bulk peat.

B. Sulfate deprivation triggers high methane production in a disturbed and rewetted coastal peatland

Abstract

In natural coastal wetlands, high supplies of marine sulfate suppress methanogenesis. Coastal wetlands are, however, often subject to disturbance by diking and drainage for agricultural use and can turn to potent methane sources when rewetted for remediation. This suggests that preceding land use measures can suspend the sulfate-related methane suppressing mechanisms. Here, we unravel the hydrological relocation and biogeochemical S and C transformation processes that induced high methane emissions in a disturbed and rewetted peatland despite former brackish impact. The underlying processes were investigated along a transect of increasing distance to the coastline using a combination of concentration patterns, stable isotope partitioning, and analysis of the microbial community structure. We found that diking and freshwater rewetting caused a distinct freshening and an efficient depletion of the brackish sulfate reservoir by dissimilatory sulfate reduction (DSR). Despite some legacy effects of brackish impact expressed as high amounts of sedimentary S and elevated electrical conductivities, contemporary metabolic processes operated mainly under sulfate-limited conditions. This opened up favorable conditions for the establishment of a prospering methanogenic community in the top 30–40 cm of peat, the structure and physiology of which resemble those of terrestrial organic-rich environments. Locally, high amounts of sulfate persisted in deeper peat layers through the inhibition of DSR, probably by competitive electron acceptors of terrestrial origin, for example Fe(III). However, as sulfate occurred only in peat layers below 30–40 cm, it did not interfere with high methane emissions on an ecosystem scale. Our results indicate that the climate effect of disturbed and remediated coastal wetlands cannot simply be derived by analogy with their natural counterparts. From a greenhouse gas perspective, the re-exposure of diked wetlands to natural coastal dynamics would literally open up the floodgates for a replenishment of the marine sulfate pool and therefore constitute an efficient measure to reduce methane emissions.

Introduction

Coastal wetlands play an important role in climate change mitigation and adaption as they can efficiently accrete organic sediments, adjust coastal elevations to sea level rise and protect low-lying areas in the hinterland. Further, while freshwater wetlands constitute the largest natural source of the greenhouse gas methane (CH_4; Zhang et al., 2017), the efficient accumulation of autochthonous C in coastal wetlands comes without the expense of high CH_4 emissions (Holm et al., 2016). Methane is a potent greenhouse gas that is formed as a terminal product of organic matter breakdown under strictly anaerobic conditions typically in the absence of electron acceptors other than CO_2 (CO_2; Segers and Kengen, 1998). In coastal environments, methane production and emission are effectively suppressed by sulfate-rich seawaters: methanogens are outcompeted by sulfate-reducing bacteria (SRB) for acetate-type precursors and hydrogen (Schönheit et al., 1982; Lovley and Klug 1983). This shifts the prevailing anaerobic C metabolic pathways from methanogenesis towards dissimilatory sulfate reduction (DSR; Martens and Berner 1974; King and Wiebe1980). In addition, sulfate (SO_4^{2-}) operates as an electron acceptor for anaerobic methane oxidation by a syntrophic consortium of anaerobic methanotrophs (ANMEs) and SRB (Iversen and Jorgensen 1985; Boetius et al., 2000). Anaerobic methane oxidation has been specifically described for brackish wetland sediments but is not exclusively confined to the utilization of sulfate as an electron acceptor (Segarra et al., 2013, 2015).

Human activities such as diking and drainage place intensive pressure on coastal landscapes with sometimes irreversible impairments of their biogeochemical cycles and ecosystem functions (Karstens et al., 2016; Zhao et al., 2016). Dikes separate coastal wetlands from resupply of sea water, and drainage for agricultural use induces the aerobic decomposition of organic-rich sediments, resulting in substantial CO_2 losses and land subsidence (Deverel and Rojstaczer 1996; Miller 2011; Deverel et al., 2016; Erkens et al., 2016). As sea levels are expected to rise, the controlled retreat from flood-prone areas becomes an essential strategy of integral coastal risk management to complement conventional technical solutions such as diking (Sánchez-Arcilla et al., 2016). Rewetting may re-establish the ability of abandoned coastal wetlands to efficiently accrete organic matter under anaerobic conditions and represents a promising management technique to reverse land surface subsidence caused by drainage-induced peat oxidation (Deverel et al., 2016; Erkens et al., 2016). Moreover, while freshwater wetlands may become methane sources upon rewetting (Wilson et al., 2009; Vanselow-Algan et al., 2015; Franz et al., 2016; Hemes et al., 2018), sulfate-rich seawater could potentially reduce post-rewetting methane release in coastal wetlands. However, recent work on a degraded brackish peatland has revealed high post-rewetting CH_4 emissions (Hahn et al., 2015; Koebsch et al., 2015) and methanogen abundance (Wen et al., 2018), thereby challenging the common notion of coastal wetlands as negligible methane emitters. In fact, diking and the drainage-rewetting cycle may induce hydrological shifts and biogeochemical transformation processes that are so far not well understood. In particular, the transformation and/or relocation of the marine sulfate reservoir in the sediments of diked wetlands are of vital importance to understand the implications of anthropogenic intervention on coastal wetland biogeochemistry and to better constrain the climate effect of coastal wetland remediation.

Here, we investigate the mechanisms that allow for high methane production in disturbed and remediated coastal wetlands. We therefore address the fate of brackish compounds and the emerging S and C transformation processes in a rewetted, freshwater-fed peatland that was naturally exposed to episodic intrusions from the Baltic Sea. In the past, the peatland had been subject to intense human intervention including diking and drainage for agricultural use. After rewetting by freshwater-flooding, the site turned into a strong methane source. The underlying hydrological and biogeochemical processes were investigated along a brackish–terrestrial transect that spans between 300 and 1500m in distance from the coastline using hydrogeochemical element patterns, stable isotope biogeochemistry, and microbiological analyses.

The specific goals were to

– retrace the marine legacy effect remaining after diking and freshwater rewetting in the peat pore space using salinity, the isotope composition of water, and a suite of inert dissolved constituents that may be indicative for the intermingling of brackish and terrestrial waters.

– track the fate of Baltic Sea-derived sulfate and uncover potential S transformation pathways using concentration patterns, stable isotope measurements of pore water SO_4^{2-} ($\delta^{34}S$ and $\delta^{18}O$), and solid S compounds as well as the bacterial community structure.

– describe evolving methane cycling processes using concentration and stable isotope measurements of CH_4 ($\delta^{13}C$, $\delta^{2}H$) and dissolved inorganic C (DIC, $\delta^{13}C$) as well as the abundance and community structure of methane-cycling microbes.

We hypothesized the marine legacy effect to be represented by a lateral gradient in electrical conductivity (EC) and pore water sulfate along the brackish–terrestrial transect. We further expected increasing terrestrial impact to promote the deprivation of the brackish sulfate pool and to induce complementary patterns of methane production.

Materials and methods

Study site and sampling design

The study site is part of the nature reserve “Heiligensee und Hütelmoor”, a 490ha coastal peatland complex located in NE Germany directly at the SW Baltic coast with an elevation between -0.3 and +0.7m above sea level (Dahms, 1991) (latitude 54° 12′, longitude 12° 10′ Fig. B-1). Climate is transitional maritime with continental influence from the east. The area receives a mean annual precipitation of 645 mm with a mean annual temperature of 9.2 °C (reference period 1982–2011, data from the German Weather Service, DWD). Peat formation was initiated by the Littorina Sea transgression and the postglacial sea level rise around 5400 BC. Presently, the Hütelmoor is fed by a 15 km^2 forested catchment dominated by gley over fine sands. Originally, the fen exhibited 0.2–2.3 m deep layers of sulfidic reedsedge peat underlain by Late Weichselian sands over impermeable till (Voigtländer et al., 1996; Bohne and Bohne, 2008). A total of 40 years of drainage for grassland use caused severe degradation of the peat, which was recently identified as sapric Histosol (Koebsch et al., 2013). Since the rewetting by flooding in 2010 through the construction of a weir at the outflow of the catchment, more than 80 % of the area has been permanently inundated with freshwater from the surrounding forest catchment (Miegel et al., 2016). Current vegetation of the Hütelmoor is dominated by patches of competitive emergent macrophytes such as reed and sedges (*Phragmites australis* (Cav.) Trin. ex Steud and *Carex acutiformis* Ehrh.) that increasingly supersede species indicative for brackish conditions (*Bolboschoenus maritimus* (L.) Palla, *Schoenoplectus tabernaemontani* (C. C. Gmel.) Palla) (Koch et al., 2017).

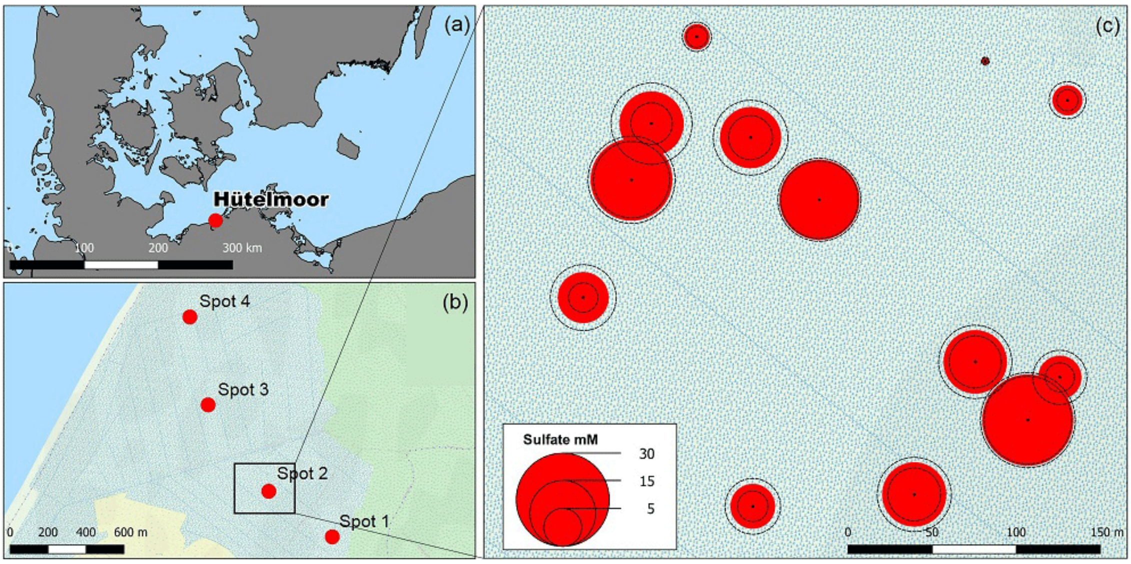


Figure B-1 – (a) The study site Hütelmoor is located directly at the southwestern Baltic coast at an altitude between -0.2 and +0.2 m a.s.l. In its pristine state, the site was exposed to episodic brackish water intrusion by storm surges. (b) Profiles of sediments and pore waters were taken along a transect *with 300–1500 m distance to the coastline. Deviations of the transect from the straight normal to the Baltic coastline arose due to the restricted accessibility of the site. (c) A former study located close to spot 2 in the center of the current sampling transect revealed high porewater sulfate concentrations in 30–60 cm below the surface with annual means up to 24 ± 3 mM (red circles indicate annual means while dashed circle lines represent the standard deviation over the year). Map data are copyrighted under OpenStreetMap contributors and available from https://www.openstreetmap.org (last access: 23 November 2016).*

Under natural coastal dynamics, the Hütelmoor is episodically flooded by storm surges. Low outflow and high evapotranspiration rates promote brackish conditions. Major brackish water intrusions were reported for 1904, 1913, 1949, 1954, and 1995 (Bohne and Bohne, 2008) though flooding frequency is reduced since the site was diked in 1903. Additional brackish input occurs through underground flow and atmospheric deposition as well as through high water situations at the Baltic Sea when backwater of the interconnected Warnow River delta enters the fen. However, potential brackish water entry paths other than storm surges have revealed a negligible effect on peat salinity (Selle et al., 2016). The last flooding event in 1995 raised EC in the drainage ditches up to 8 mS cm^{-1}, but the EC decreased to the pre-flooding level of 2 mS cm^{-1} within the following 5 years (Bohne and Bohne, 2008).

Samples were collected at four spots along a transect with increasing distance to the Baltic Sea (300–1500 m, Fig. B-1b) within 2 weeks in October/November 2014. The transect included the area of a former study which revealed high concentrations of brackish SO_4^{2-} with annual means up to 23.7 ± 3.2 mM (Koch, unpublished, Fig. B-1c). At the time of sampling, water depth above the peat surface spanned from 9 to 19 cm, which presented the lowest range within the seasonal water level fluctuation. Sampling depth ranged from 45 to 65 cm, which was in most cases sufficient to cover the full peat depth including the underlying mineral soil.

Pore water analysis

Pore waters were collected from distinct depth below the surface (cmb.s.f.) with a stainless-steel push-point sampler attached to a syringe to draw the sample from a distinct penetration depth. Temperature, pH, EC, and salinity were measured directly after sampling (Sentix 41 pH probe and a TetraCon 325 conductivity-measuring cell attached to a WTW multi 340i handheld; WTW, Weilheim). Samples were filtered (0.45 µm membrane syringe filters) *in situ* and transferred without headspace into vials (except for dissolved CH_4). Vials had been previously preconditioned with 1 M HCl and subsequent 1 M NaOH and were filled with a compound-specific preservative (see below).

Dissolved CH_4 concentration was measured with the headspace approach. Therefore, 5 mL of pore water was transferred into 12 mL septum-capped glass vials under atmospheric pressure. Before taking them to the field, the sampling vials were flushed with Ar and filled with 500 µL saturated HgCl solution to prevent further biological activity. After sampling, the punctuated septum was covered with lab foil and the vials were stored upside down to minimize CH_4 loss. Headspace gas concentrations after equilibration were measured in duplicates with an Agilent 7890A gas chromatograph equipped with a flame ionization detector and with a carbon plot capillary column or HP-Plot Q (Porapak Q) column. Helium was used as the tracer gas. Gas sample analyses were performed after calibration of the gas chromatograph with gas standards (accuracy > 98.5 %). The measured headspace CH_4 concentration was then converted into dissolved CH_4 concentration using the temperature-corrected solubility coefficient (Wilhelm et al., 1977).

Samples for anion concentrations (SO_4^{2-}, Cl^-, Br^-) were filled in 20 mL glass vials preserved with 1 mL 5 % ZnAc solution to prevent sulfide oxidation. Anion concentrations were analyzed by ion chromatography (Thermo Scientific Dionex) in a continuous flow of 9 mM NaCO3 eluent in an IonPac AS9-HC 4 column, partly after dilution of the sample. The device was calibrated with NIST standard reference material solutions freshly prepared before each run to span the concentration ranges of the (diluted) samples. Reproducibility between sample replicates was smaller than ± 5 %.

For H_2S analysis, pore water was filled into 5mL polypropylene vials and preserved with 0.25 mL 5 % ZnAc solution. H_2S concentration was measured photometrically (Specord 40, Analytic Jena) using the methylene blue method (Cline, 1969).

The metal and total dissolved S (TSdiss) concentrations were analyzed by ICP-OES (inductively coupled plasma optical emission spectrometry, iCAP 6300 DUO Thermo Fisher Scientific) after appropriate dilution. Since high amounts of dissolved organic carbon (DOC) may cause severe interferences in the ICP-OES element measurements, samples were boiled in Teflon beakers with 65 % HNO_3 and subsequently 19 % HCl prior to analysis. The accuracy and precision were routinely checked with the certified CASS standards as described previously (Kowalski et al., 2012). The residual, non-specified S fraction (ResS resulting from the difference between TSdiss, H_2S, and SO_4^{2-} is suggested to consist primarily of dissolved organic S, polysulfides, and S intermediates. $\delta^{13}C$ and δD values of methane were analyzed using the gas chromatography–combustion technique (GC-C) and the gas chromatography–high-temperature-conversion technique (GC-HTC). The gas was directly injected in a gas chromatograph Agilent 7890 (Agilent Technologies, Germany), the peaks were separated using a CP-PoraBOND Q GC column (50 m

× 0.32 mm × 5 µm, isotherm 60 °C, Varian). Methane was quantitatively converted to the analysis gases CO_2 and H_2 in the GC–Isolink interface (Thermo Finnigan, Germany) and directly transferred via open split interface (ConFlo IV, Thermo Finnigan, Germany). The $\delta^{13}C$ and δD values of both gases were then measured with the isotope ratio mass spectrometer MAT 253 (Thermo Finnigan, Germany). Results for $\delta^{13}C$ ratios of methane are given in the usual δ notation versus the Vienna PeeDee Belemnite (VPDB) standard. δD-CH_4 ratios were referenced to the Vienna Standard Mean Ocean Water (V-SMOW).

The carbon isotope values ($\delta^{13}C$) of DIC were measured from a HgCl-preserved solution using a Thermo Finnigan MAT 253 gas mass spectrometer coupled to a Thermo Electron Gas Bench II via a Thermo Electron ConFlo IV split interface. NBS19 and LSVEC were used to scale the isotope measurements to the VPDB standard. Based on replicate measurements of standards, reproducibility was better than ±0.1 ‰ (Winde et al., 2014).

For the determination of sulfate isotope signatures, dissolved sulfate was precipitated with 5 % barium chloride as barium sulfate (Böttcher et al., 2007). After precipitation the solid was filtered, washed and dried, and further combusted in a Thermo Flash 2000 EA elemental analyzer that was connected to a Thermo Finnigan MAT 253 gas mass spectrometer via a Thermo Electron ConFlo IV split interface with a precision of better than ± 0.2 ‰. Isotope ratios are converted to the Vienna Canyon Diablo Toilite (VCDT) scale (Mann et al., 2009). For oxygen isotope analyses, $BaSO_4$ was decomposed by means of pyrolysis in silver cups using a high-temperature conversion elemental analyzer (HTO-, Hekatech, Germany) connected to an isotope gas mass spectrometer (Thermo Finnigan MAT 253) (Kornexl et al., 1999). The calibration took place via the reference materials IAEA-SO-5 and IAEA-SO-6 and $^{18}O/^{16}O$ values were referenced to the V-SMOW standard. Replicate measurements agreed within ± 0.5 ‰. Stable oxygen (O) isotope measurements of pore waters were conducted using a CRDS system (Picarro L2140-i) versus the V-SMOW standard. International V-SMOW, SLAP, and GISP in addition to in-house standards were used to scale the isotope measurements.

Sediment analysis

Intact peat cores were collected with a perspex liner (ID: 59.5 mm) and subsequently punched out layer by layer. The peat section protruding from the end of the liner was divided into three subsamples for the analysis of (i) total reduced inorganic S (TRIS), (ii) total solid S (TSsolid) and reactive iron, and (iii) the microbial community structure. In order to minimize oxygen contamination, the outer layer of the peat core was omitted and subsamples were immediately packed. The aliquot for TRIS analysis was preserved with 1:1 (v/v) 20 % ZnAc. Subsamples for microbial analysis were immediately stored in RNAlater stabilization solution to preserve DNA. A second core was taken for the analysis of water content and dry bulk density. TSsolid and TRIS samples were frozen within 8h after collection. Aliquots for TSsolid elemental analysis were further freeze-dried and milled in a planet-ball mill. TSsolid contents were analyzed by means of dry combustion using an Eltra CS-2000 after combustion at 1250 °C. The device was previously calibrated with a certified coal standard and precision is better than ± 0.02 %.

TRIS fractions were determined by a two-step sequential extraction of iron monosulfides and pyrite (Fossing and Jørgensen, 1989). The acid volatile sulfur (AVS) fraction was extracted by the reaction with 1 M HCl for 1 h under a continuous stream of di-nitrogen gas. The H_2S released was quantitatively precipitated as ZnS and then determined spectrophotometrically with a Specord 40 spectrophotometer following the method of Cline (1969). Chromium-reducible sulfur (CRS; essentially pyrite; FeS_2), was extracted with hot acidic Cr(II)chloride solution. For $\delta^{34}S$ analysis in different TRIS fractions the ZnS was converted to Ag_2S by addition of 0.1 M $AgNO_3$ solution with subsequent filtration, washing, and drying of the $AgNO_3$ precipitate as described by Böttcher and Lepland (2000). The non-specified solid S fraction, resulting from the difference between TSsolid, CRS, and AVS, was suggested to present primarily organic-bond S (orgS). The $\delta^{34}S$ composition of this residual fraction was measured from the washed and dried solid residue after the Cr(II) extraction step via C-IRmMS following the approach of Passier (1999). Reactive iron was extracted from freeze-dried sediments by the reaction with a 1 M HCl solution for 1 h (e.g., Canfield, 1989).

Iron was determined as Fe^{2+} after reduction with hydroxylamine hydrochloride via spectrophotometry using ferrozine as the complexing agent (Stookey, 1970). Reactive iron here is considered to be the sum of those iron fractions that may still react with dissolved sulfide. This fraction includes iron(III)oxyhydroxides and acid volatile sulfide (AVS, essentially FeS) as well as a very minor contribution from dissolved Fe^{2+} in the pore water (Canfield, 1989).

Microbial community analysis

Genomic DNA of 0.2–0.3 g of sediment was extracted with the EURx soil DNA kit (Roboklon, Berlin, Germany) according to manufactory protocols. DNA concentrations were quantified with a Nanophotometer® P360 (Implen GmbH, Munich, DE) and Qubit® 2.0 fluorometer (Thermo Fisher Scientific, Darmstadt, Germany) according to the manufactory protocols.

The 16S rRNA gene for bacteria was amplified with the primer combination S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 (Herlemann et al., 2011). The 16S rRNA gene for archaea was amplified with the primer combination S-D-Arch-0349-a-S-17 and S-D-Arch-0786-a-A-20 (Takai and Horikoshi, 2000). The primers were labeled with unique combinations of bar codes. The PCR mix contained 1× PCR buffer (Tris • Cl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$; pH 8.7) (Qiagen, Hilden, Germany), 0.5 µM of each primer (Biomers, Ulm, Germany), 0.2 mM of each deoxynucleoside (Thermo Fisher Scientific, Darmstadt, Germany), and 0.025UµL^{-1} hot start polymerase (Qiagen, Hilden, Germany). The thermocycler conditions were 95 °C for 5 min (denaturation), followed by 40 cycles of 95 °C for 1 min (denaturation), 56°C for 45s (annealing), and 72°C for 1min and 30s (elongation), concluded with a final elongation step at 72 °C for 10min. PCR products were purified with a Hi Yield® Gel/PCR DNA fragment extraction kit (Süd-Laborbedarf, Gauting, Germany) according to the manufactory protocol. PCR products of three individual runs per sample were combined. PCR products of different samples were pooled in equimolar concentrations and compressed to a final volume of 10 µL with a concentration of 200 ng µL^{-1} in a vacuum centrifuge concentrator plus (Eppendorf, Hamburg, Germany). Individual samples were sequenced in duplicates.

The sequencing was performed on an Illumina MiSeq sequencer by the company GATC. The library was prepared with the MiSeq Reagent Kit V3 for 2 × 300 bp paired-end reads according to the manufactory protocols. For better performance due to different sequencing length we used 15 % PhiX control v3 library.

The quality of the sequences was checked using the fastqc tool (FastQC A Quality Control tool for High Throughput Sequence Data; http://www.bioinformatics.babraham.ac. uk/projects/fastqc/, last access: 29 June 2018; by Andrews, 2010). Raw sequence reads were demultiplexed, and bar codes were removed with the CutAdapt tool (Martin, 2011). The subsequent steps included merging of reads using overlapping sequence regions (PEAR; Zhang et al., 2014), standardizing the nucleotide sequence orientation, and trimming and filtering of low-quality sequences (Trimmomatic) (Bolger et al., 2014). After quality filtering, chimera were removed by the ChimeraSlayer tool of the QIIME pipeline. Subsequently, sequences were clustered into operational taxonomic units (OTUs) at a nucleotide cutoff level of 97 % similarity and singletons were automatically deleted. To reduce noise in the dataset, sequences with relative abundances below 0.1 % per sample were also removed. All archaeal libraries contained at least > 18 500 sequences, while bacterial libraries contained at least > 12 500 sequences. OTUs were taxonomically assigned employing the GreenGenes database 13.05 (McDonald et al., 2012) using the QIIME pipeline (Caporaso et al., 2010).

Representative sequences of OTUs were checked for correct taxonomical classification by phylogenetic tree calculations in the environment. Relative abundance of sequences related to known methanogens, anaerobic methanotrophs (ANME), and sulfate reducers were used to project microbial depth profiles. Sequences have been deposited at NCBI under the BioProject PRJNA356778 with the sequence read archive accession numbers SRR5118134-SRR5118155 for bacterial and SRR5119428-SRR5119449 for archaeal sequences, respectively.

Results

Porewater geochemical patterns and pore water isotope composition

Substantial amounts of dissolved salts with EC maxima of up to 11.5 mS cm^{-1} occurred at peat depths below 30 cm b.s.f. (centimeters below surface; Fig. B-2a, Table B-S1) and corresponded with brackish pore water proportions of up to 60 % (based on Baltic Sea salinity reported by Feistel et al., 2010). Only at spot 1, with the greatest distance to the coastline, did lower EC values (max. 3.4 mS cm^{-1}) indicate minor brackish pore water proportions (5 %–6 %). At the other three spots, EC values were similar, i.e., exhibited no lateral salinity graduation along the remaining Baltic Sea–freshwater transect.

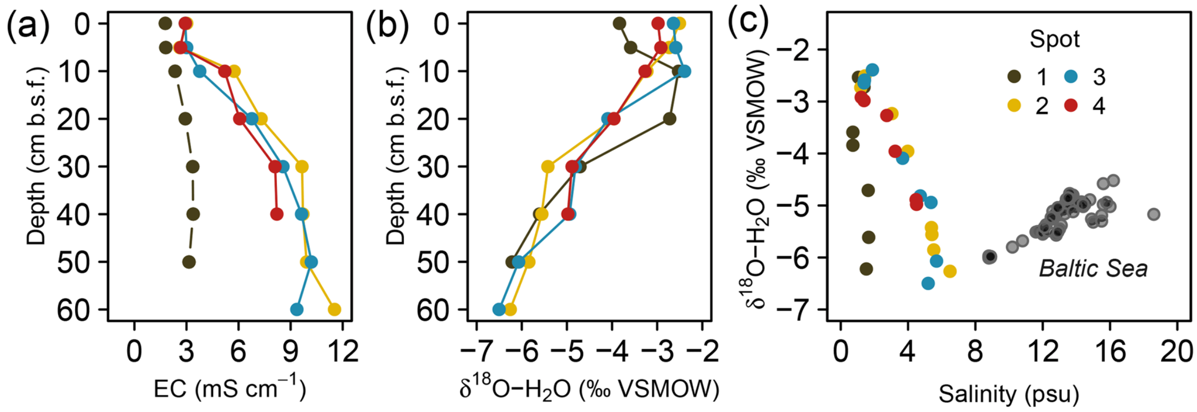


Figure B-2 – Depth distribution of electrical conductivity (EC, a) and pore water O isotope composition (b). Panel (c) depicts a scatter plot of pore water O isotope composition and salinity. Grey transparent dots in (c) represent a common positive $\delta_{18}O$–H_2O vs. salinity relationship derived from a sampling campaign of Baltic Sea surface water (Westphal, unpublished).

Vertical trends in pore water stable O isotope composition were similar for all spots and complementary to the salinity and EC patterns with an upwards increase from 60 to 10cm b.s.f. (Fig. B-2b). The resulting salinity–$\delta^{18}O$ relationship was negative (except for the low salinity gradient at freshwater spot 1) and thus inverse to the common salinity–$\delta^{18}O$ trend characteristic for Baltic coastal waters (Fig. B-2c). This suggests that distribution patterns of salinity have formed independently from evaporative fractionating effects observed in the top pore water layers.

The pore water geochemistry in the peatland was increasingly diversified with depth: while the top 10 cm b.s.f. was comparatively homogenous across all spots, specific patterns evolving from diagenetic differences emerged primarily in deeper pore waters. Principal component analysis (Fig. B-3) revealed the pore water geochemical composition below 10 cm b.s.f. to be constrained by two major components that evolved in opposed lateral directions and, in concert, explained 90 % of the variation in pore water composition. A distinct gradient associated with a depth increase in EC and the associated conservative ions (Cl^-, Na^+, Br^-) suggests a persistent brackish impact at spots 2, 3, and 4 (first principal component, explained 55 % of the total variation). Only at spot 1, farthest away from the coastline, was the EC increase with depth minute. This EC gradient was further negatively correlated with pH, indicating a general decrease in pH with depth and the highest pH values around 7.0 at spot 1. A second distinct lateral gradient was delineated by the concentrations of dissolved Fe, Mn, DIC, and Ca, which occurred in higher abundances at spots 1 and 2 closest to the upstream terrestrial catchment boundary (second principal component, explained 35 % of the total variation). Such a lateral shift in pore water geochemistry is probably related to the supply of mineral solutes from terrestrial inflow. In this regard, the pore water composition of spot 2 united the elevated supply in mineral compounds from terrestrial inflow with persisting remnants of former brackish impact.

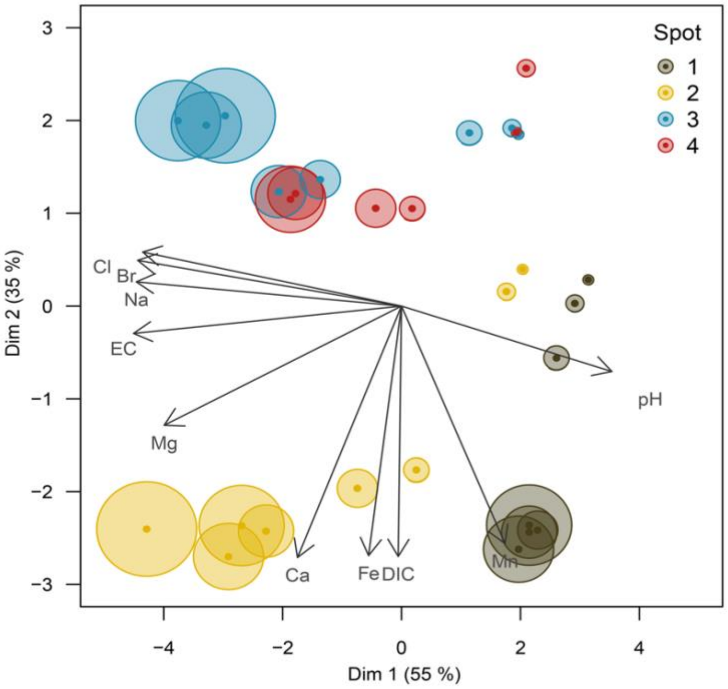


Figure B-3 – Principal component biplot of pore water geochemical patterns within the peatland. Different colors indicate different sampling locations within the brackish–freshwater continuum with spot 1 closest to the freshwater catchment and spot 4 closest to the Baltic Sea. The size of the data points scales with *sampling depth (smallest points indicate surface patterns; largest points indicate pore water composition at 60 cm in depth).*

Sulfur speciation, S isotope patterns, and sulfate reducing communities

We found distinct differences in the S biogeochemical patterns across spots indicating different sulfate supply and transformation processes along the terrestrial–brackish continuum (Fig. B-4). In the following, we structured the results spot-wise according to the specific S regime and address first spot 1 (low solid sulfur and low sulfate), then spots 3 and 4 (high solid sulfur and low sulfate), and finally spot 2 (high solid sulfur and partially high sulfate concentrations).

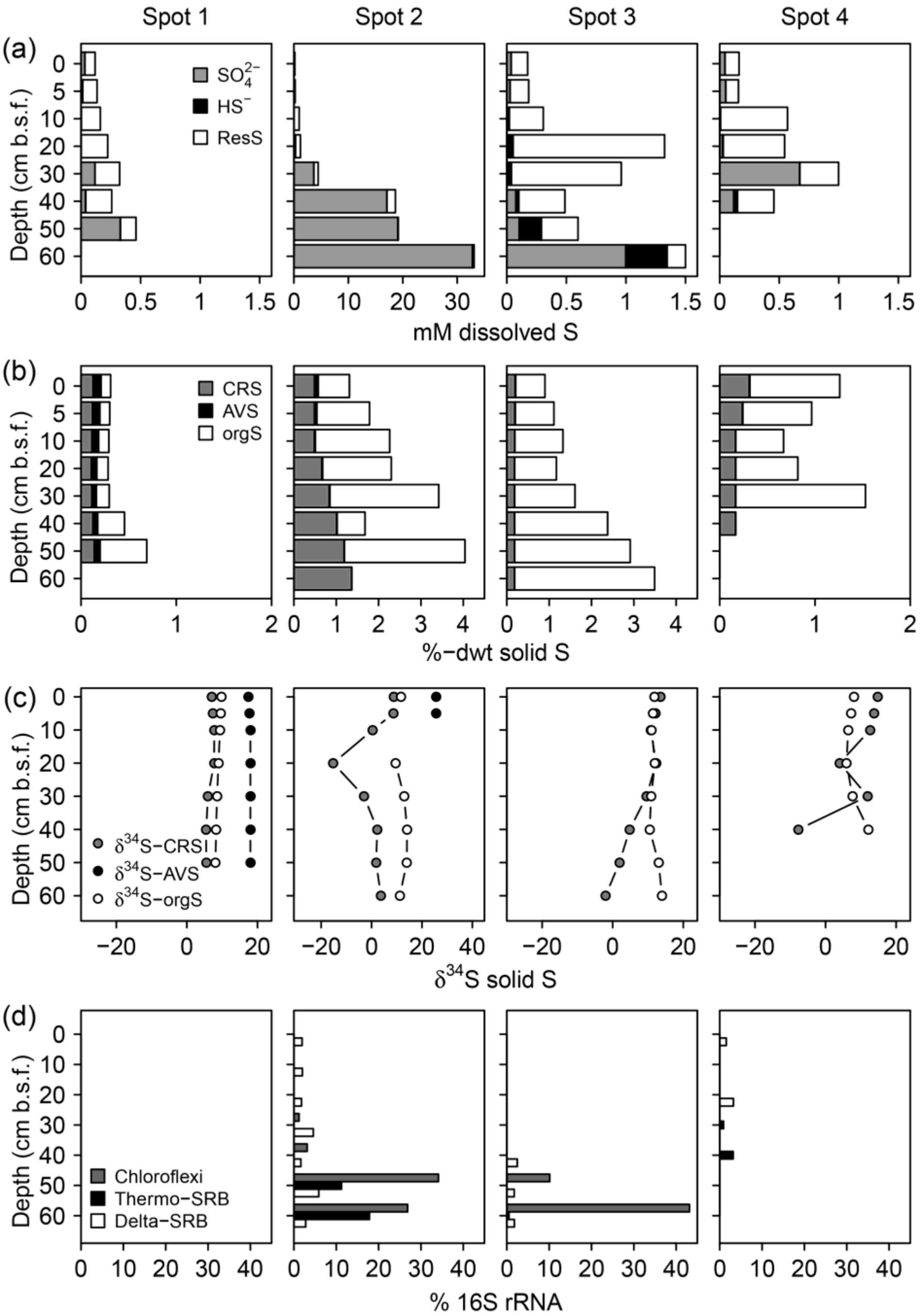


Figure B-4 – Speciation of dissolved (a) and solid (b) S compounds, S isotope composition of solid S compounds (c), and average relative abundances of sulfate-reducing bacteria (SRB, d). $\delta^{34}S$ and $\delta^{18}O$ ratios of SO_4^{2-} are displayed in Fig. B-6a. The residual dissolved S (ResS in a) refers to a non-specified S fraction resulting from the difference between total dissolved S, H_2S, and SO_4^{2-}. ResS is most likely composed of dissolved organic S, polysulfides, and S intermediates. Solid S fractions (b) include iron monosulfide operationally defined as acid volatile sulfur (AVS), pyrite extracted as chromium-reducible sulfur (CRS), and a residual fraction suggested to consist primarily of organic S (orgS). $\delta^{34}S$ at AVS could only be measured at spot 1 and the top of spot 2. SRB were extracted from two replicates of the 16S rRNA bacterial community sequencing and are assigned to the Deltaproteobacteria (Delta-SRB) and the Nitrospirae phyla (genus Thermodesulfovibrionaceae – Thermo-SRB). Chloroflexi Dehalococcoides (Chloroflexi) have not been assigned to SRB in the classical sense; however, they could be potentially involved in S metabolism (Wasmund et al., 2016). Note the different x axis scales.

Spot 1

Spot 1 characterized by low salinities and mineral inflow from the near freshwater catchment exhibited the lowest sulfate concentrations of ≤0.3 mM. H_2S concentrations hardly exceeded the detection limit (~1 µM, Fig. B-4). Sulfate made up only a small proportion of the TSdiss pool, thereby indicating a higher abundance of a non-specified dissolved S fraction, probably composed of dissolved organic S, polysulfides, and S intermediates. In addition, the abundance of solid S was lowest at spot 1 (≤0.7 %dry weight (dwt) TSsolid). Among solid S compounds, organic-bond S constituted the dominant solid S fraction (0.1 to 0.5 %dwt) with relatively stable $\delta^{34}S$ ratios (+8.1 ‰ and +9.8 ‰). Pyrite contents (measured as CRS) were low despite abundant pore water Fe and available solid iron (Fig. B-5). Only at spot 1 did we find a low though consistent abundance of iron monosulfides (0.1 %dwt, measured as AVS). Biogeochemical turnover processes here might operate under sulfate-limited conditions resulting in lower sedimentary S contents and accumulation of iron monosulfides. In correspondence with the low sulfate contents, no sulfate-reducing bacteria occurred at spot 1.

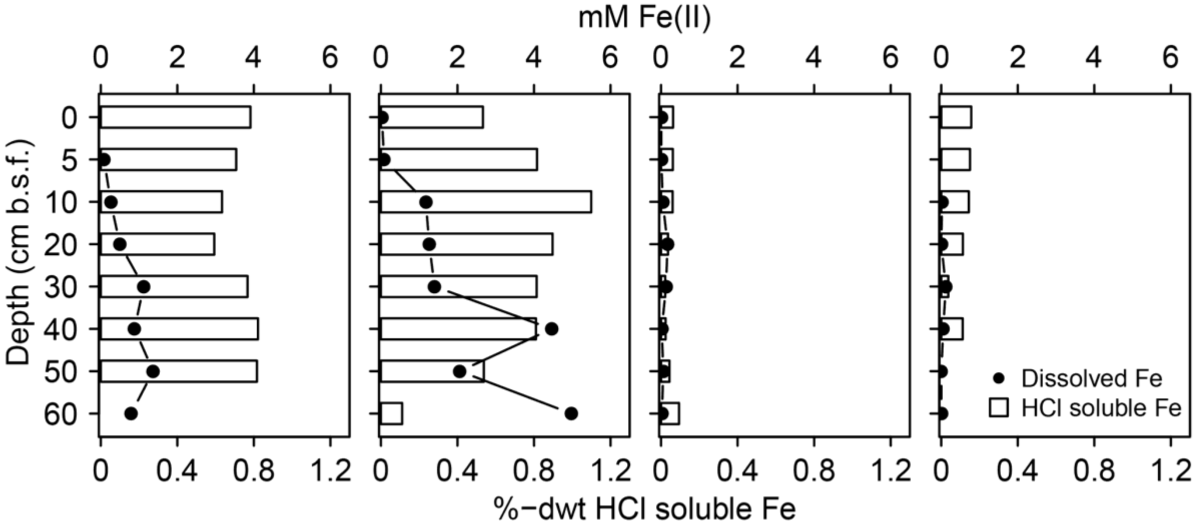


Figure B-5 – Mobile Fe species. Available solid iron was extracted as HCl soluble iron from the sediment matrix and is composed of iron mono-sulfide and non-sulfidized ferric Fe.

Spots 3 and 4

Despite the persisting brackish impact found in the deeper pore waters of spots 3 and 4 closest to the Baltic Sea, we found hardly any pore water sulfate in the top 20 cm b.s.f. ($\leq$0.1 mM) and only moderate SO_4^{2-} levels down to 30 cm b.s.f. (0.1–1 mM). H_2S abundance was essentially restricted to the depth at spot 3 (up to 347 µM).

Low porewater sulfate concentrations prevented $\delta^{34}S$ measurements at the majority of the data points. However, the single $\delta^{34}S$ value of +86.4 ‰ measured at 60 cm b.s.f. of spot 3 (Fig. B-6a) indicated a remarkable ^{34}S enrichment in relation to Baltic Sea water SO_4^{2-} (+21 ‰; Böttcher et al., 2007). Sulfur isotope fractionation to this extent is likely to result from a superposition of enzymatic kinetic fractionation associated with a reservoir effect and constitutes striking isotopic evidence for the exhaustion of the brackish sulfate pool by intense DSR (Hartmann and Nielsen 2012). Despite missing isotope measurements, it is likely that the low sulfate concentrations at the remaining depth sections of spot 3 and along the depth profile of spot 4 result from the same intense sulfate reduction processes.

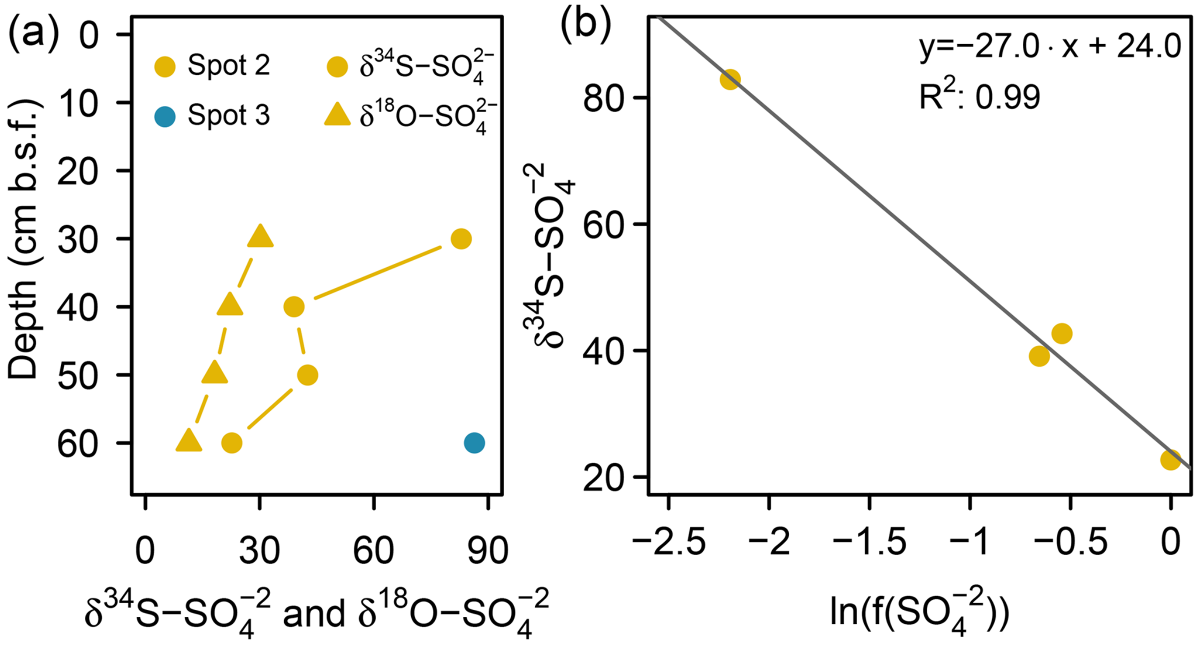


Figure B-6 – (a) S and O isotope composition of sulfate. Sufficient SO_4^{2-} for $\delta^{34}S$ and $\delta^{18}O$ ratio analysis was only available at the bottom of spot 2 and spot 3 (here only $\delta^{34}S$). (b) Rayleigh plot for measured SO_4^{2-} depletion at spot 2.

We measured high amounts of TSsolid (up to 3.5 %dwt) at the depth of spot 3. In both, spots 3 and 4, organic-bond S constituted the dominant solid S fraction (0.5 to 3.3 %dwt) but was completely missing at the depth of spot 4. Pyrite was less abundant (0.2–0.3 %dwt) and exhibited a wide range of δ^{34}S ratios (−15 ‰ to +11 ‰). As pyrite δ^{34}S ratios essentially reflect the isotopic signature of the sulfide pool derived from DSR (Butler et al., 2004; Price and Shieh 1979), the found variation in pyrite δ^{34}S ratios reflected different stages of a reservoir effect that varies in response to the openness of the system (i.e., connectivity to the sea). In correspondence with the exhaustion of the brackish sulfate pool, the relative abundance of SRB was generally small (< 5 %) and most likely substrate-limited. SRB were from the Deltaproteobacteria class and the Thermodesulfovibrionaceae genus of the Nitrospirae phylum. With 40 % relative abundance, Chloroflexi of the class Dehalococcoidetes represented the dominating bacterial group at the 1 mM SO_4^{2-} concentration depth of spot 3.

Spot 2

At spot 2 – the interface between brackish impact and mineral inflow from the freshwater catchment – we found a sharp rise in SO_4^{2-} concentration from ≤0.3 mM at the top 20 cm up to 32.8 mM at 60 cm b.s.f. The latter exceeded the quantities expected from marine supply (Kwiecinski 1965; Feistel et al., 2010) by a factor of 8. The pronounced concentration gradient at spot 2 was associated with a remarkable variation in the stable isotope composition showing a downcore decrease in δ^{34}S–SO_4^{2-} from +82.9 ‰ to +22.7 ‰ and a decrease in δ^{18}O–SO_4^{2-} from +30 ‰ to +11 ‰ (Fig. B-6a). δ^{34}S values > +80 ‰ at 30 cm b.s.f. of spot 2 suggest the brackish sulfate pool in the top pore waters to be microbially exhausted under the same reservoir effect as in spots 3 and 4. The δ^{18}O and δ^{34}S ratios of excess SO_4^{2-} in 60 cm b.s.f. (δ^{34}S: +22.7 ‰; δ^{18}O: +11.4 ‰) corresponded well with modern-day seawater SO_4^{2-} (δ^{34}S: +21 ‰; δ^{18}O: +9 ‰; Böttcher et al., 2007). Altogether, the sharp sulfate concentration and isotope gradients at spot 2 could demonstrate the entire spectrum of sulfate speciation from the persistence of a marine sulfate reservoir at 60 cm b.s.f. towards progressing sulfate depletion in the upper peat layers.

To test this hypothesis, we applied a closed-system (Rayleigh-type) model (Eq. 1, Mariotti et al., 1981) to the data from spot 2 and gained an estimate for the δ^{34}S ratios of the initial SO_4^{2-} reservoir ($\delta^{34}\mathrm{S}_{\mathrm{SO}^{2-}_{4,initial}}$) and the kinetic isotope enrichment factor ε:

$$\delta^{34}\mathrm{S}_{\mathrm{SO}^{2-}_{4,depth}} - \delta^{34}\mathrm{S}_{\mathrm{SO}^{2-}_{4,initial}} = \varepsilon \ln\left(f\mathrm{SO}^{2-}_{4,depth}\right). \tag{1}$$

Here $\delta^{34}\mathrm{S}_{\mathrm{SO}^{2-}_{4,depth}}$ represents the S isotope values measured in specific depths of spot 2, and fSO_4^{2-} $_{,depth}$ constitutes the fraction of remaining pore water SO_4^{2-} in relation to the initial sulfate reservoir (32.8 mM SO_4^{2-}, measured in 60 cm b.s.f. at spot 2). The fit through four data points (R^2: 0.99;

$p > 0.05$) revealed the $\delta^{34}S$ ratios of the initial SO_4^{2-} reservoir (+24 ‰) to be close to the ^{34}S signature of the Baltic Sea (Fig. B-6b). The isotopic offset is within the uncertainty of the estimate. The isotope enrichment factor ε was estimated to be −27 ‰, which is within the range reported for DSR in laboratory studies with pure cultures (Kaplan and Rittenberg, 1964; Canfield, 2001; Sim et al., 2011) and in the field (Habicht and Canfield, 1997; Böttcher et al., 1998).

The pronounced sulfate distribution patterns at spot 2 went along with the highest amounts of pyrite (0.5–1.4 %dwt). Pyrite contents increased with depth and partially exceeded the amounts of organic-bond S. The patterns in pyrite $\delta^{34}S$ ratios did not correspond with the vertical trend in sulfate availability. Instead, $\delta^{34}S$ values were lowest in 20 cm b.s.f. (−15 ‰) and stabilized around +2 ‰ below.

Interestingly, at peak sulfate supply of spot 2, the relative abundance of Deltaproteobacteria did not exceed 5 %. Instead, the SRB community at depth was dominated by the Thermodesulfovibrionaceae genus that contributed up to 21 % of all bacterial 16S rRNA sequences. Likewise, with spot 3, Chloroflexi of the class Dehalococcoidetes also represented the dominating bacterial group at the depth of spot 2.

Dissolved methane concentrations, isotopic signature, and methanogenic communities

Measured pore water CH_4 concentrations were up to 643 µM with equivocal vertical patterns across spots (Fig. B-7a), reflecting the methane-specific spatial variability that evolves from small-scale heterogeneity in production and consumption processes and from ebullitive release events (Chanton et al., 1989; Whalen 2005). Here, we use the isotope composition of CH_4 (Fig. B-7b) and DIC (Fig. B-7c) to provide a clearer (and probably more robust) indication for patterns of methanogenesis and methanotrophy. Methanogenesis is a highly fractionating process: in comparison to the starting organic material ($\delta^{13}C$ ~−27 ‰ in this study), the produced CH_4 is distinctively ^{13}C-depleted, whilst at the same time, CO_2 becomes considerably enriched in ^{13}C (Whiticar et al., 1986). In this respect, high $\delta^{13}C$-DIC ratios up to +4.2 ‰ suggest intense methanogenic (i.e., ^{13}C-DIC fractionating) processes in 20–40 cm b.s.f., whereas DIC on top was comparatively depleted in ^{13}C as is characteristic for methane oxidation in the aerated surface layers. $\delta^{13}C$-DIC ratios below 40 cm b.s.f. converged towards the isotopic signature of bulk organic C (−26 ‰).

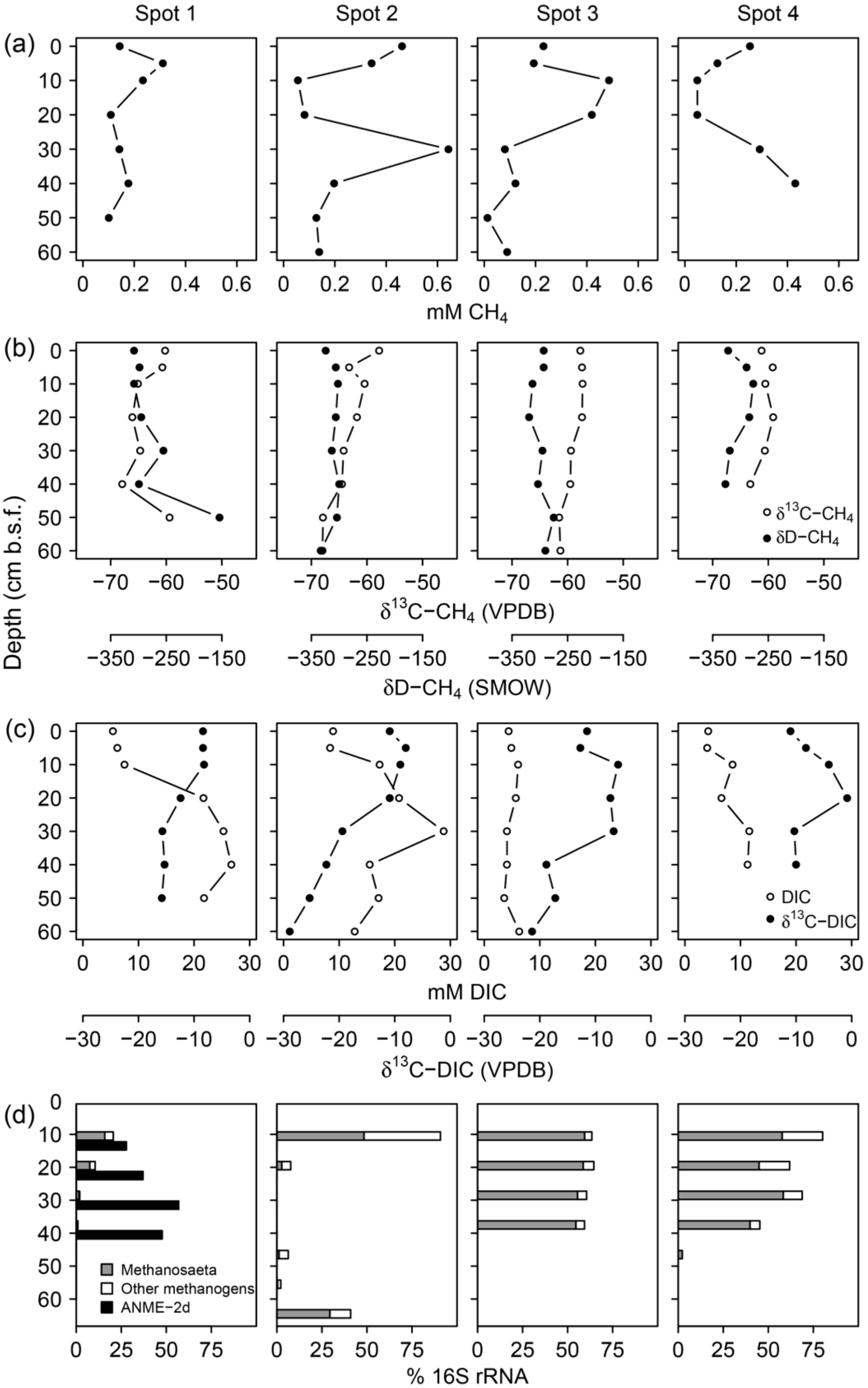


Figure B-7 – Concentration patterns and isotope ratios for CH_4 *(a, b) and DIC (c), as well as average relative abundances of methanogens and methanotrophs (d).*

At spot 2, we found the most pronounced downward drop in δ^{13}C-DIC ratios with a minimum of −23.9 ‰ in 60 cm b.s.f. This pattern coincided with a consistent downward decrease in δ^{13}C-CH_4 ratios from −57 ‰ to −68 ‰ and suggests that methanogenesis operates under higher ^{13}C fractionation associated with thermodynamically less favorable conditions at the bottom of spot 2. δD ratios of methane did not exhibit a concurrent increase but varied unrelated to δ^{13}C-CH_4 ratios in a range between −333 ‰ and −275 ‰. Based on the C and D isotopic ratio threshold raised by Whiticar (1986), acetate fermentation revealed to be the dominant methane production pathway at our study site (Fig. B-8). A concurrent rise in both δD-CH_4 and δ^{13}C-CH_4 ratios at the depth of spot 1 suggests a shift towards dominating CO_2 reduction and/or an increase in methanotrophy.

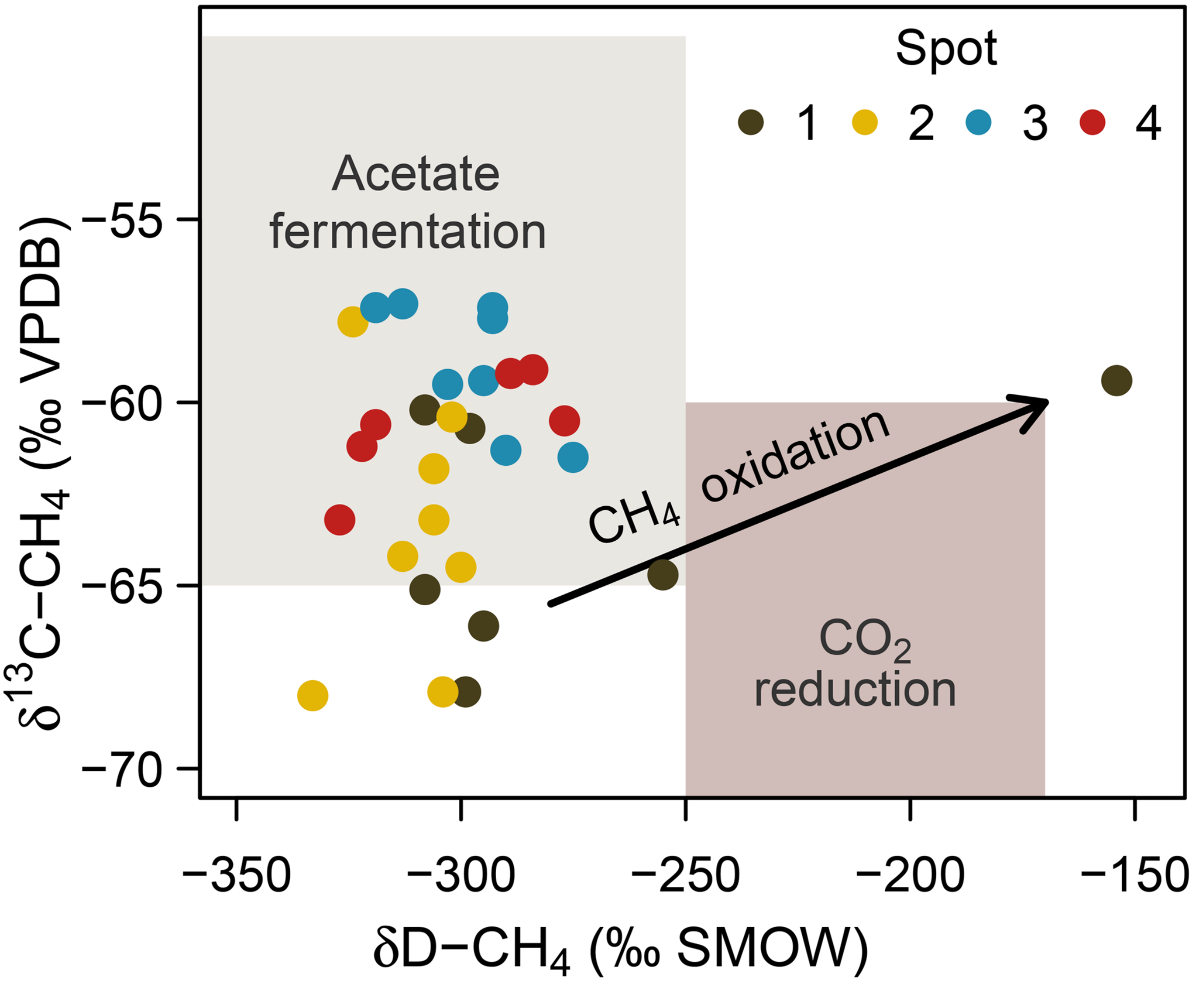


Figure B-8 – Projection of the CH_4 stable isotope composition to differentiate dominating methanogenic pathways and methanotrophy. Isotope thresholds to confine methanogenic pathways are based on Whiticar et al., (1986). The concurrent increase in δ^{13}C-CH_4 and δD-CH_4 values at spot 1 suggests a downward shift towards increasing CO_2 reduction or CH_4 oxidation rates at depth.

Together with high δ^{13}C-DIC ratios in the upper parts of the peat, 16S rRNA sequences related to methanogens (Fig. B-7d) provided further evidence for intensive methane production. At spot 2, we found the largest divergence with 90 % methanogen-related sequences at the surface while in deeper regions (10–50 cm b.s.f.) less than 7 % of the archaeal domain could be attributed to methanogens. Surprisingly, at 60 cm b.s.f. of spot 2, methanogen percentages increased abruptly up to 41 % despite high relative abundances of SRB. Spot 1 exhibited the lowest methanogen proportions, which decreased from 21 % at the top down to 1 % in 50 cm b.s.f.

The methanogen community was mostly dominated by *Methanosaeta*, an obligate acetotrophic archaea genus that thrives in terrestrial organic-rich environments. The *Methanosaeta* proportion usually scaled with the methanogen percentage and contributed 70 %–100 % to the methanogenic community. Whilst methanogenic pathways derived from the isotopic composition of CH_4 can be obscured by the fractionating effect of methanotrophy, the phylogenetic structure of the methanogenic community provided clear evidence for acetate fermentation as the prevailing methanogenic pathway in most of the peatland.

Sequences related to aerobic methanotrophs of the genus Methylosinus were only found at 30 cm b.s.f. in spot 4 representing approximately 1.5 % of all bacterial sequences (data not shown). Aerobic methanotrophs were underrepresented in our dataset. Consistent with the concurrent depth increase in $\delta^{13}C$-CH_4 and δD-CH_4, spot 1 (Fig. B-8), situated at the fringe of the freshwater catchment, exhibited high abundances of anaerobic methanotrophs of the ANME-2d clade that are so far implicated to use NO^{3-} (Raghoebarsing et al., 2006) and/or Fe(III) (Ettwig et al., 2016) as electron acceptors.

Discussion

Porewater biogeochemical patterns

Overall, the pore water geochemistry of the Hütelmoor was characterized by two different aspects: a legacy effect delineated by the lateral brackish–terrestrial continuum below 20 to 30 cm in depth and an overlying recent layer representing the uniform freshwater regime induced by rewetting.

Despite a continuous groundwater inflow from the forested catchment (Miegel et al., 2016), relics of former brackish and mineral terrestrial inflow are preserved in the deeper layers of the peat body. This is exemplified by high pore water EC values that exceeded those reported directly after the last brackish water intrusion event in 1995 (Bohne and Bohne, 2008). In fact, discharge within the peatland is channeled through rapid flow in the drainage ditches while water movement within the interstitial peat body seems to be mostly restricted to vertical exchange processes (evaporation, precipitation) with minor lateral flow (Selle et al., 2016). Therefore, we assume that drainage-induced hydrological alterations reinforced the segregation of the peat pore matrix from subsurface lateral exchange. This would allow for the preservation of residual signals in deeper pore waters and would further confine contemporary biogeochemical transformation processes to the recycling of autochthonous matter. The new top freshwater layer, established after flooding in 2010, overprints lateral differences along the brackish–fresh continuum and unifies the upper pore water geochemistry in the entire peatland.

Sulfur transformation

Along the entire brackish–terrestrial transect, virtually no sulfate was abundant in the newly developed fresh pore water layer at the top 20 cm. However, distinct differences in sulfur speciation across spots were preserved below 20 cm b.s.f. and seemed to reflect the gradual exposure to former brackish intrusion and terrestrial inflow.

Spot 1 appeared to be virtually unaffected by any brackish impact with biogeochemical turnover processes operating under sulfate-limited conditions. Low sedimentary S contents and the accumulation of iron monosulfides as representative for freshwater environments are strong points for this conclusion.

Also, at spots 3 and 4, contemporary biogeochemical processes essentially operated under sulfate-limited conditions, although these areas had been exposed to flooding from the nearby Baltic Sea. High sedimentary S concentrations in conjunction with the ^{34}S composition of the remaining sulfate suggest that the brackish sulfate reservoir has been essentially exhausted through DSR with the produced sulfide being either incorporated as diagenetically derived S in organic compounds or precipitated as ^{34}S-enriched pyrite minerals (Brown and MacQueen 1985; Hartmann and Nielsen 2012). Hence, if diking of coastal wetlands prevents the replenishment of the brackish sulfate reservoir, the latter can be almost completely consumed through DSR as has been demonstrated by the Rayleigh distillation model. The rapid exhaustion of the brackish sulfate reservoir is likely to be reinforced in coastal peatlands where vast amounts of C compounds constitute an extensive electron donor supply for DSR.

Prevalent sulfate limitation at spots 1, 3, and 4 was reflected by the virtual absence of the sulfate-reducing microbial community. Interestingly, minor remnants of the brackish sulfate pool (1 mM SO_4^{2-}) at the depth of spot 3 were associated with 40 % relative abundance of Chloroflexi of the class Dehalococcoidetes. Genomes of this group in marine sediments have been shown to code for *dsrAB* genes (Wasmund et al., 2016). Through their ability to reduce sulfite they may be involved in S redox cycling. Indeed, further research is required to better establish their function in the S cycle.

S geochemistry at spot 2, which unites the effects of brackish water intrusion with mineral inflow of terrestrial origin, differed substantially from the other spots with remarkably high sulfate concentrations (33 mM) at depth. The mineral impact from terrestrial inflow was not only reflected by high concentrations of dissolved constituents (Fe, DIC, Mg, Ca, Mn) but also by high contents of labile iron minerals and dissolved ferrous iron. Interactions with poorly ordered ferric hydroxides can supply Fe(III) as a competitive electron acceptor next to sulfate (Postma and Jakobsen 1996) and may, therefore, inhibit the efficient microbial reduction of the brackish sulfate reservoir. Amorphous ferric hydroxides effectively suppressed DSR in a recently rewetted Baltic coastal wetland (Virtanen et al., 2014). In our study, high contents of labile iron minerals and dissolved ferrous iron at the depth of spot 2 coincided with a high abundance of Thermodesulfovibrionaceae and a concurrently minor occurrence of Deltaproteobacteria. Recent in vitro experiments suggest Thermodesulfovibrionaceae can utilize ferric iron as an electron acceptor next to sulfate (Fortney et al., 2016). Indeed, the demonstration of Fe(III) reduction by Thermodesulfovibrionaceae under *in situ* conditions is currently still pending. Nevertheless, high contents of labile iron minerals, the remarkable accumulation of pore water iron, and the absence of typical iron reducers (Geobacteraceae, Peptococcaceae, Shewanellaceae, Desulfovibrionaceae, Pelobacteraceae) could suggest Thermodesulfovibrionaceae prefer Fe(III) as an electron acceptor over sulfate. Thus, the unique SO_4^{2-} concentration patterns at spot 2 may be attributed to the inhibited microbial consumption of the brackish sulfate reservoir caused by the delivery of alternative electron acceptors from the nearby freshwater catchment.

Altogether, our results demonstrate the potential fate of the brackish sulfate reservoir in coastal wetlands under closed system conditions caused by diking. Microbial transformation processes have decoupled the sulfate distribution patterns from the relic brackish impact and have caused marked differences in contemporary sulfate biogeochemistry: on the one hand, DSR exhausted the brackish sulfate reservoir in wide parts of the peatlands, whereas, on the other hand, the preferential consumption of competitive electron acceptors from terrestrial origin allowed for the local accumulation of large sulfate concentrations. Indeed, these relic signals of brackish–terrestrial intermixing are constrained to the deeper pore water regions below 30 cm b.s.f. as recent rewetting measures established a homogeneous freshwater regime in the top layers of the entire peatland.

Methane production and consumption

δ^{13}C-DIC ratios and a thriving methanogenic community indicate the establishment of distinct methane production zones in the recently formed freshwater layer across the entire peatland. In line with the prevalent freshwater characteristics of the newly formed pore water layer, the methanogen community was dominated by *Methanosaeta*, an obligate acetotrophic genus typical of terrestrial organic-rich environments. Indeed, thermodynamically favorable methanogenic conditions were confined to the top layers since isotopic evidence and archaeal distribution patterns indicate a downward shift towards non-fractionating metabolic processes (Barker, 1936; Lapham et al., 1999) at the bottom. This vertical transition was most pronounced at spot 2, probably indicating a potential suppression of methanogenesis by high concentrations of sulfate and labile ferric iron compounds at depth.

Surprisingly, we observed mutual coexistence of SRB (22 % of all bacterial sequences) and methanogens (> 40 % of all archaeal sequences) at high SO_4^{2-} concentrations (32.8 mM) in 60 cm b.s.f. at spot 2. Simultaneous methanogenesis and DSR have been reported under the abundance of methanol, trimethylamine, or methionine as methanogenic precursors (Oremland and Polcin 1982). However, the concurrent high abundance of *Methanosaeta* (30 %) at the depth of spot 2 suggests competitive consumption of acetate by both SRB and methanogens. Although Liebner et al. (2015) emphasized the relevance of community structure with regard to prevailing methanogenic pathways, total abundance data could potentially yield more insight into this issue.

Sequences related to aerobic methanotrophs of the genus Methylosinus were only found at 30 cm b.s.f. in spot 4, representing approximately 1.5 % of all bacterial sequences (data not shown). The phenomenon of a lagged reestablishment of methanotrophs in comparison to methanogens after rewetting in this particular peatland is addressed in another publication (Wen et al., 2018).

Despite the overlap of methane production zones anticipated from $\delta^{13}C$-DIC ratios with sulfate reduction zones, we could not find evidence for the syntrophic consortium of anaerobic methanotrophs (ANME) and sulfate reducers that is commonly associated with the anaerobic oxidation of methane coupled to sulfate reduction (AOM-SR) in marine environments (Boetius et al., 2000). However, we cannot exclude that AOM-SR is driven by archaea that are so far not known for this function. One potential candidate phylum is the Bathyarchaota that have been shown to encode an untypical version of the functional gene for methane production and consumption (methyl coenyzme M reductase subunit A, *mcrA*; Evans et al., 2015). These archaea dominated spot 2 with 48 %–97 % relative sequence abundance of the archaeal community between 10 and 60 cm (data not shown).

While we cannot supply microbial evidence for AOM-SR, high abundances of anaerobic methanotrophs of the ANME-2d clade at spot 1 suggest anaerobic methane oxidation coupled to electron acceptors of terrestrial origin. Methanotrophs of the ANME-2d clade are so far known to utilize NO^{3-} (Raghoebarsing et al., 2006) and ferric iron (Ettwig et al., 2016) as electron acceptors, both of which were abundant at the respective spot. This observation is further supported by the trend in $\delta^{13}C$-CH_4 and δD-CH_4 that potentially indicates a downward increase in methanotrophy at spot 1. The biogeochemical characteristics at this very location result most likely from formerly drier conditions due to slightly higher elevation in combination with prevalent inflow from the nearby forest catchment.

Our results demonstrate how rewetting of a coastal peatland established a distinct freshwater regime in the upper pore water layers, which, in conjunction with prevalent anaerobic conditions and a vast stock of labile C compounds, offers favorable conditions for intense methane production and explains the high methane emissions reported in Hahn et al. (2015) and Koebsch et al. (2015). As intense methane production was confined to the upper pore water layers in the entire peatland, it did not interfere with high sulfate concentrations locally preserved as the legacy of former brackish impact in the bottom. Instead, isotopic and microbial evidence suggested mineral compounds of terrestrial origin to constitute an electron acceptor for anaerobic methane oxidation, which is an often neglected – though it is an important process in freshwater environments (Segarra et al., 2015). Our results indicate that this process can also occur in disturbed coastal peatlands. Indeed, the quantitative effects of anaerobic methane consumption on methane emissions in coastal and/or rewetted peatlands need to be addressed in future studies.

Conclusions

In this study, we investigated the biogeochemical and hydrological mechanisms that turn disturbed and remediated coastal peatlands into strong methane sources. Our study demonstrates how human intervention overrides the sulfate-related processes that suppress methane production and thereby suspends the natural mechanisms that mitigate greenhouse gas emissions from coastal environments. Hence, the climate effect of disturbed and remediated coastal wetlands cannot simply be derived by analogy with their natural counterparts. Instead, human alterations form new transient systems where relic brackish signals intermingle with recent freshwater impacts. The evolving biogeochemical patterns overprint naturally established gradients formed, for instance, by the distance to the coastline. In particular, the decoupling of sulfate abundance from salinity is of high practical relevance for greenhouse gas inventories that establish methane emission factors based on the empirical relation to salinity as an easily accessible proxy for sulfate concentrations.

Coastal environments are subject to particular pressure by high population density while at the same time their potential as coastal buffer zones is moving more and more into the focus of policy makers and land managers. From a greenhouse gas perspective, the exposure of diked wetlands to natural coastal dynamics would literally open the floodgates for a replenishment of the marine sulfate pool and constitute an efficient measure to reduce methane emissions. However, in practice, this option has to be weighed against concurrent land use aspects.

Acknowledgements

This work was supported by the DFG Research Training Group BALTIC TRANSCOAST (grant DFG GRK 2000) and the Helmholtz Terrestrial Environmental Observatories (TERENO) Network. This is BALTIC TRANSCOAST publication number GRK2000/0023. Franziska Koebsch was supported by the Helmholtz Association of German Research Centers through the Helmholtz Postdoc Program (grant PD-129) and the Helmholtz Climate Initiative REKLIM (Regional Climate Change). Franziska Koebsch was further supported by the European Social Fund (ESF) and the Ministry of Education, Science and Culture of Mecklenburg-West Pomerania within the scope of the project WETSCAPES (ESF/14-BM-A55-0030/16). Torsten Sachs and Susanne Liebner were each supported by a Helmholtz Young Investigators Group (grants VH-NG-821 and VH-NG-919). Biogeochemical and stable isotope work was supported by the Leibniz Institute for Baltic Sea Research (IOW). We wish to express our gratitude to Lisa Kretzschmann, Anke Saborowski, and Simon Strunk for their commitment to field work under tough conditions. Bennet Juhls and Simon Strunk have helped with map creation. The study would not have been possible without the laboratory and bioinformatics support by Andrea Gottsche, Anke Saborowski, Lisa Kretzschmann, Anne Köhler, Birgit Plessen, Vera Winde, Fabian Horn, Xi Wen, and Heiko Baschek.

References

Andrews, S.: FastQC: a quality control tool for high throughput sequence data, available at: http://www.bioinformatics.babraham. ac.uk/projects/fastqc (last access: 29 June 2018), 2010.

Barker, H. A.: On the biochemistry of the methane fermentation, Arch. Mikrobiol., 7, 404–419, 1936.

Boetius, A., Ravenschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O.: A marine microbial consortium apparently mediating anaerobic oxidation of methane, Nature, 407, 623–626, 2000.

Bohne, B. and Bohne, K.: Monitoring zum Wasserhaushalt einer auf litoralem Versumpfungsmoor gewachsenen Regenmoorkalotte – Beispiel Naturschutzgebiet "Hütelmoor" bei Rostock, Aspekte der Geoökologie, Weißensee Verlag, Berlin, Germany, 2008.

Bolger, A. M., Lohse, M., and Usadel, B.: Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics, 30, 2114–2120, 2014.

Böttcher, M., Brumsack, H.-J., and Dürselen, C.-D.: The isotopic composition of modern seawater sulfate: I. Coastal waters with special regard to the North Sea, J. Marine Syst., 67, 73–82, 2007.

Böttcher, M. E. and Lepland, A.: Biogeochemistry of sulfur in a sediment core from the west-central Baltic Sea: evidence from stable isotopes and pyrite textures, J. Marine Syst., 25, 299–312, 2000.

Böttcher, M. E., Brumsack, H.-J., De Lange, G. J., and Robertson, A.: Sulfate reduction and related stable isotope (^{34}S, ^{18}O) variations in interstitial waters from the Eastern Mediterranean, 365–376, 1998.

Brand, W. A. and Coplen, T. B.: Stable isotope deltas: tiny, yet robust signatures in nature, Isot. Environ. Healt. S., 48, 393–409, 2012.

Brown, K. and MacQueen, J.: Sulphate uptake from surface water by peat, Soil Biol. Biochem., 17, 411–420, 1985.

Butler, I. B., Böttcher, M. E., Rickard, D., and Oldroyd, A.: Sulfur isotope partitioning during experimental formation of pyrite via the polysulfide and hydrogen sulfide pathways: implications for the interpretation of sedimentary and hydrothermal pyrite isotope records, Earth Planet. Sc. Lett., 228, 495–509, 2004.

Canfield, D. E.: Reactive iron in marine sediments, Geochim. Cosmochim. Ac., 53, 619–632, 1989.

Canfield, D. E.: Isotope fractionation by natural populations of sulfate-reducing bacteria, Geochim. Cosmochim. Ac., 65, 1117–1124, 2001.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Pena, A. G., Goodrich, J. K., and Gordon, J. I.: QIIME allows analysis of high-throughput community sequencing data, Nat. Methods, 7, 335–336, 2010.

Chanton, J. P., Martens, C. S., and Kelley, C. A.: Gas transport from methane-saturated, tidal freshwater and wetland sediments, Limnol. Oceanogr., 34, 807–819, 1989.

Cline, J. D.: Spectrophotometric determination of hydrogen sulfide in natural waters 1, Limnol. Oceanogr., 14, 454–458, 1969.

Dahms, P.: Studie Wasserregulierung Hütelmoor, Universität Rostock, Fachbereich Landeskultur und Umweltschutz, Fachgebiet Kulturtechnik, Rostock, Germany, 1991.

Deverel, S. J. and Rojstaczer, S.: Subsidence of agricultural lands in the Sacramento-San Joaquin Delta, California: Role of aqueous and gaseous carbon fluxes, Water Resour. Res., 32, 2359–2367, 1996.

Deverel, S. J., Ingrum, T., and Leighton, D.: Present-day oxidative subsidence of organic soils and mitigation in the SacramentoSan Joaquin Delta, California, USA, Hydrogeol. J., 24, 569–586, 2016.

Erkens, G., van der Meulen, M. J., and Middelkoop, H.: Double trouble: subsidence and CO_2 respiration due to 1000 years of Dutch coastal peatlands cultivation, Hydrogeol. J., 24, 551–568, 2016.

Ettwig, K. F., Zhu, B., Speth, D., Keltjens, J. T., Jetten, M. S., and Kartal, B.: Archaea catalyze iron-dependent anaerobic oxidation of methane, P. Natl. Acad. Sci. USA, 113, 12792–12796, 2016.

Evans, P. N., Parks, D. H., Chadwick, G. L., Robbins, S. J., Orphan, V. J., Golding, S. D., and Tyson, G. W.: Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics, Science, 350, 434–438, 2015.

Feistel, R., Weinreben, S., Wolf, H., Seitz, S., Spitzer, P., Adel, B., Nausch, G., Schneider, B., and Wright, D. G.: Density and Absolute Salinity of the Baltic Sea 2006–2009, Ocean Sci., 6, 3–24, https://doi.org/10.5194/os-6-3-2010, 2010.

Fortney, N., He, S., Converse, B., Beard, B., Johnson, C., Boyd, E. S., and Roden, E.: Microbial Fe (III) oxide reduction potential in Chocolate Pots hot spring, Yellowstone National Park, Geobiology, 14, 255–275, 2016.

Fossing, H. and Jørgensen, B. B.: Measurement of bacterial sulfate reduction in sediments: evaluation of a single-step chromium reduction method, Biogeochemistry, 8, 205–222, 1989.

Franz, D., Koebsch, F., Larmanou, E., Augustin, J., and Sachs, T.: High net CO_2 and CH_4 release at a eutrophic shallow lake on a formerly drained fen, Biogeosciences, 13, 3051–3070, https://doi.org/10.5194/bg-13-3051-2016, 2016.

Habicht, K. S. and Canfield, D. E.: Sulfur isotope fractionation during bacterial sulfate reduction in organic-rich sediments, Geochim. Cosmochim. Ac., 61, 5351–5361, 1997.

Hahn, J., Köhler, S., Glatzel, S., and Jurasinski, G.: Methane exchange in a coastal fen in the first year after flooding-a systems shift, PloS one, 10, e0140657, 2015.

Hartmann, M. and Nielsen, H.: $\delta^{34}S$ values in recent sea sediments and their significance using several sediment profiles from the western Baltic Sea, Isot. Environ. Healt. S., 48, 7–32, 2012.

Hemes, K. S., Chamberlain, S. D., Eichelmann, E., Knox, S. H., and Baldocchi, D. D.: A biogeochemical compromise: The high methane cost of sequestering carbon in restored wetlands, Geophys. Res. Lett., 45, 6081–6091, 2018.

Herlemann, D. P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J., and Andersson, A. F.: Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea, ISME J., 5, 1571–1579, 2011.

Holm, G. O., Perez, B. C., McWhorter, D. E., Krauss, K. W., Johnson, D. J., Raynie, R. C., and Killebrew, C. J.: Ecosystem level methane fluxes from tidal freshwater and brackish marshes of the Mississippi River Delta: Implications for coastal wetland carbon projects, Wetlands, 36, 401–413, 2016.

Iversen, N. and Jorgensen, B. B.: Anaerobic methane oxidation rates at the sulfate-methane transition in marine sediments from Kattegat and Skagerrak (Denmark) 1, Limnol. Oceanogr., 30, 944–955, 1985.

Kaplan, I. and Rittenberg, S.: Microbiological fractionation of sulphur isotopes, Microbiology, 34, 195–212, 1964.

Karstens, S., Buczko, U., Jurasinski, G., Peticzka, R., and Glatzel, S.: Impact of adjacent land use on coastal wetland sediments, Sci. Total Environ., 550, 337–348, 2016.

King, G. M. and Wiebe, W.: Regulation of sulfate concentrations and methanogenesis in salt marsh soils, Estuar. Coast. Mar. Sci., 10, 215–223, 1980.

Koch, M., Koebsch, F., Hahn, J., and Jurasinski, G.: From meadow to shallow lake: Monitoring secondary succession in a coastal fen after rewetting by flooding based on aerial imagery and plot data, Mires Peat, 19, 1–17, 2017.

Koebsch, F., Glatzel, S., and Jurasinski, G.: Vegetation controls methane emissions in a coastal brackish fen, Wetl. Ecol. Manag., 21, 323–337, 2013.

Koebsch, F., Jurasinski, G., Koch, M., Hofmann, J., and Glatzel, S.: Controls for multi-scale temporal variation in ecosystem methane exchange during the growing season of a permanently inundated fen, Agr. Forest Meteorol., 204, 94–105, 2015.

Kornexl, B. E., Werner, R. A., and Gehre, M.: Standardization for oxygen isotope ratio measurement – still an unsolved problem, Rapid Commun. Mass Sp., 13, 1248–1251, 1999.

Kowalski, N., Dellwig, O., Beck, M., Grunwald, M., Dürselen, C.-D., Badewien, T. H., Brumsack, H.-J., van Beusekom, J. E., and Böttcher, M. E.: A comparative study of manganese dynamics in the water column and sediments of intertidal systems of the North Sea, Estuar. Coast. Shelf S., 100, 3–17, 2012.

Kwiecinski, B.: The sulfate content of Baltic water and its relation to the chlorinity, Deep-Sea Res., 12, 797–804, 1965.

Lapham, L., Proctor, L., and Chanton, J.: Using respiration rates and stable carbon isotopes to monitor the biodegradation of orimulsion by marine benthic bacteria, Environ. Sci. Technol., 33, 2035–2039, 1999.

Liebner, S., Ganzert, L., Kiss, A., Yang, S., Wagner, D., and Svenning, M. M.: Shifts in methanogenic community composition and methane fluxes along the degradation of discontinuous permafrost, Front. Microbiol., 6, 1–10, 2015.

Lovley, D. R. and Klug, M. J.: Sulfate reducers can outcompete methanogens at freshwater sulfate concentrations, Appl. Environ. Microb., 45, 187–192, 1983.

Mann, J. L., Vocke Jr., R. D., and Kelly, W. R.: Revised δ^{34}S reference values for IAEA sulfur isotope reference materials S-2 and S-3, Rapid Commun. Mass Sp., 23, 1116–1124, 2009.

Mariotti, A., Germon, J. C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., and Tardieux, P.: Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes, Plant Soil, 62, 413–430, 1981.

Martens, C. S. and Berner, R. A.: Methane production in the interstitial waters of sulfate-depleted marine sediments, Science, 185, 1167–1169, 1974.

Martin, M.: Cutadapt removes adapter sequences from high-throughput sequencing reads, EMBnet. Journal, 17, 10–12, 2011.

McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., Andersen, G. L., Knight, R., and Hugenholtz, P.: An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea, ISME J., 6, 610–618, 2012.

Miegel, K., Graeff, T., Selle, B., Salzmann, T., Franck, C., and Bronstert, A.: Untersuchung eines renaturierten Niedermoores an der mecklenburgischen Ostseeküste – Teil I: Systembeschreibung und hydrologische Grundcharakterisierung, Hydrol. Wasserbewirts., 60, 242–258; https://doi.org/10.5675/HyWa_2016,4_1, 2016.

Miller, R. L.: Carbon gas fluxes in re-established wetlands on organic soils differ relative to plant community and hydrology, Wetlands, 31, 1055–1066, 2011.

Oremland, R. S. and Polcin, S.: Methanogenesis and sulfate reduction: competitive and noncompetitive substrates in estuarine sediments, Appl. Environ. Microb., 44, 1270–1276, 1982.

Passier, H. F., Böttcher, M. E., and De Lange, G. J.: Sulphur enrichment in organic matter of eastern Mediterranean sapropels: a study of sulphur isotope partitioning, Aquat. Geochem., 5, 99–118, 1999.

Postma, D. and Jakobsen, R.: Redox zonation: equilibrium constraints on the Fe(III)/SO4-reduction interface, Geochim. Cosmochim. Ac., 60, 3169–3175, 1996.

Price, F. T. and Shieh, Y.: Fractionation of sulfur isotopes during laboratory synthesis of pyrite at low temperatures, Chem. Geol., 27, 245–253, 1979.

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

Sánchez-Arcilla, A., García-León, M., Gracia, V., Devoy, R., Stanica, A., and Gault, J.: Managing coastal environments under climate change: Pathways to adaptation, Sci. Total Environ., 572, 1336–1352, 2016.

Schönheit, P., Kristjansson, J. K., and Thauer, R. K.: Kinetic mechanism for the ability of sulfate reducers to out-compete methanogens for acetate, Arch. Microbiol., 132, 285–288, 1982.

Segarra, K., Schubotz, F., Samarkin, V., Yoshinaga, M., Hinrichs, K., and Joye, S.: High rates of anaerobic methane oxidation in freshwater wetlands reduce potential atmospheric methane emissions, Nat. Commun., 6, 7477–7488, 2015.

Segarra, K. E., Comerford, C., Slaughter, J., and Joye, S. B.: Impact of electron acceptor availability on the anaerobic oxidation of methane in coastal freshwater and brackish wetland sediments, Geochim. Cosmochim. Ac., 115, 15–30, 2013.

Segers, R. and Kengen, S.: Methane production as a function of anaerobic carbon mineralization: a process model, Soil Biol. Biochem., 30, 1107–1117, 1998.

Selle, B., Graeff, T., Salzmann, T., Oswald, S. E., Walther, M., and Miegel, K.: Untersuchung eines renaturierten Mooreinzugsgebiets an der mecklenburgischen Ostseeküste – Teil II: Salzdynamik und Wasserhaushalt, Hydrol. Wasserbewirts., 60, 259–268, https://doi.org/10.5675/HyWa_2016,4_2, 2016.

Sim, M. S., Bosak, T., and Ono, S.: Large sulfur isotope fractionation does not require disproportionation, Science, 333, 74–77, 2011.

Stookey, L. L.: Ferrozine – a new spectrophotometric reagent for iron, Anal. Chem., 42, 779–781, 1970.

Takai, K. and Horikoshi, K.: Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes, Appl. Environ. Microb., 66, 5066–5072, 2000.

Vanselow-Algan, M., Schmidt, S. R., Greven, M., Fiencke, C., Kutzbach, L., and Pfeiffer, E.-M.: High methane emissions dominated annual greenhouse gas balances 30 years after bog rewetting, Biogeosciences, 12, 4361–4371, https://doi.org/10.5194/bg-12-4361-2015, 2015.

Virtanen, S., Simojoki, A., Hartikainen, H., and Yli-Halla, M.: Response of pore water Al, Fe and S concentrations to waterlogging in a boreal acid sulphate soil, Sci. Total Environ., 485, 130–142, 2014.

Voigtländer, U., Schmidt, J., and Scheller, W.: Pflege- und Entwicklungsplan NSG Heiligensee und Hütelmoor, 1996.

Wasmund, K., Cooper, M., Schreiber, L., Lloyd, K. G., Baker, B. J., Petersen, D. G., Jørgensen, B. B., Stepanauskas, R., Reinhardt, R., and Schramm, A.: Single-cell genome and group-specific *dsrAB* sequencing implicate marine members of the class Dehalococcoidia (Phylum Chloroflexi) in Sulfur Cycling, MBio, 7, 1–15, e00266-00216, 2016.

Wen, X., Unger, V., Jurasinski, G., Koebsch, F., Horn, F., Rehder, G., Sachs, T., Zak, D., Lischeid, G., Knorr, K.-H., Böttcher, M. E., Winkel, M., Bodelier, P. L. E., and Liebner, S.: Predominance of methanogens over methanotrophs in rewetted fens characterized by high methane emissions, Biogeosciences, 15, 6519–6536, https://doi.org/10.5194/bg-15-6519-2018, 2018.

Whalen, S.: Biogeochemistry of methane exchange between natural wetlands and the atmosphere, Environ. Eng. Sci., 22, 73–94, 2005.

Whiticar, M. J., Faber, E., and Schoell, M.: Biogenic methane formation in marine and freshwater environments: CO_2 reduction vs. acetate fermentation – isotope evidence, Geochim. Cosmochim. Ac., 50, 693–709, 1986.

Wilhelm, E., Battino, R., and Wilcock, R. J.: Low-pressure solubility of gases in liquid water, Chem. Rev., 77, 219–262, 1977.

Wilson, D., Alm, J., Laine, J., Byrne, K. A., Farrell, E. P., and Tuittila, E. S.: Rewetting of cutaway peatlands: are we re-creating hot spots of methane emissions?, Restor. Ecol., 17, 796–806, 2009.

Winde, V., Böttcher, M., Escher, P., Böning, P., Beck, M., Liebezeit, G., and Schneider, B.: Tidal and spatial variations of DI^{13}C and aquatic chemistry in a temperate tidal basin during winter time, J. Marine Syst., 129, 396–404, 2014.

Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A.: PEAR: a fast and accurate Illumina Paired-End reAd mergeR, Bioinformatics, 30, 614–620, 2014.

Zhang, Z., Zimmermann, N. E., Stenke, A., Li, X., Hodson, E. L., Zhu, G., Huang, C., and Poulter, B.: Emerging role of wetland methane emissions in driving 21st century climate change, P. Natl. Acad. Sci. USA, 114, 9647–9652, 2017.

Zhao, Q., Bai, J., Huang, L., Gu, B., Lu, Q., and Gao, Z.: A review of methodologies and success indicators for coastal wetland restoration, Ecol. Indic., 60, 442–452, 2016.

Supplement

Table B-S1 1– Site parameters, pore water, and soil characteristics. Water level and soil depth are given in centimeters above and centimeters below surface (cm a.s.f. and cm b.s.f., respectively).

Spot	Water level cm a.s.f.	Depth cm b.s.f.	pH	Sal ppt	EC mS cm^{-1}	Cl$^-$ mM	Br$^-$ µM	Na$^+$ mM	TS$_{diss}$ mM	SO$_4^{2-}$ mM	H$_2$S µM	TS$_{solid}$ %dwt	CRS %dwt	AVS %dwt	orgS %dwt	CH$_4$ µM	DIC mM
1	14	0	6.7	0.7	1.8	11.5	19.9	9.6	0.1	0.0	1	0.3	0.1	0.1	0.1	144	5.4
		5	7.0	0.7	1.8	12.6	19.9	10.7	0.1	0.0	0	0.3	0.1	0.1	01	312	6.2
		10	7.0	1.0	2.4	14.6	19.1	10.7	0.2	0.0	3	0.3	0.1	0.1	0.1	234	7.5
		20	7.1	1.4	2.9	11.0	25.6	10.5	0.2	0.0	1	0.3	0.1	0.1	0.1	109	21.7
		30	7.1	1.6	3.4	12.5	31.9	14.1	0.3	0.1	1	0.3	0.1	0.0	0.1	143	25.3
		40	7.2	1.7	3.4	11.4	31.3	13.7	0.3	0.0	2	0.5	0.1	0.1	0.3	178	26.7
		50	7.1	1.5	3.2	12.0	38.1	13.5	0.5	0.3	0	0.7	0.1	0.1	0.5	101	21.8
2	9	0	6.9	1.4	3.0	19.3	37.0	18.2	0.2	0.0	0	1.3	0.5	0.1	0.7	462	8.9
		5	6.7	1.2	2.6	23.3	39.0	17.8	0.2	0.0	1	1.8	0.5	0.1	1.2	344	8.4
		10	7.2	3.0	5.7	37.9	46.5	32.6	1.0	0.0	6	2.3	0.5	0.0	1.8	56	17.3
		20	7.0	4.0	7.3	48.3	82.1	41.4	1.2	0.3	7	2.3	0.7	0.0	1.6	82	20.8
		30	6.5	5.4	9.7	63.7	99.8	56.5	4.5	3.7	5	3.4	0.8	0.0	2.6	643	28.8
		40	6.4	5.4	9.7	64.9	125.3	64.3	18.6	17.1	34	1.7	1.0	0.0	0.7	197	15.5
		50	6.0	5.5	9.9	67.8	129.5	61.7	18.3	19.1	61	4.0	1.2	0.0	2.8	128	17.1
		60	5.1	6.5	11.5	75.5	85.8	63.9	32.6	32.8	274	0.5	1.4	0.0	0.0	139	12.8
3	9	0	6.6	1.4	2.9	22.2	151.6	19.6	0.2	0.0	0	0.9	0.2	0.0	0.7	231	4.4
		5	6.6	1.4	3.0	22.4	49.8	20.9	0.2	0.0	1	1.1	0.2	0.0	0.9	193	4.9
		10	6.4	1.9	3.8	28.6	50.9	28.1	0.3	0.0	21	1.3	0.2	0.0	1.1	486	6.1
		20	6.1	3.7	6.8	54.5	64.9	48.3	1.3	0.0	53	1.2	0.2	0.0	1.0	420	5.7
		30	6.5	4.7	8.6	69.4	122.9	58.7	1.0	0.0	38	1.6	0.2	0.0	1.4	81	4.1
		40	5.6	5.4	9.6	87.2	156.3	55.7	0.5	0.0	25	2.4	0.2	0.0	2.2	122	4.1
		50	5.8	5.7	10.2	92.8	168.5	77.0	0.6	0.1	187	2.9	0.2	0.0	2.7	13	3.6
		60	6.0	5.2	9.4	77.6	181.6	70.9	1.5	1.0	347	3.5	0.2	0.0	3.3	89	6.3
4	19	0	6.6	1.4	2.9	20.5	159.4	19.2	0.2	0.0	1	1.3	0.3	0.0	0.9	254	4.2
		5	6.7	1.2	2.7	22.6	49.4	19.8	0.2	0.1	0	1.0	0.2	0.0	0.7	127	4.0
		10	6.6	2.7	5.2	37.7	48.4	33.1	1.0	0.0	7	0.7	0.2	0.0	0.5	48	8.6
		20	7.2	3.2	6.1	52.3	84.9	44.3	1.0	0.0	5	0.8	0.2	0.0	0.7	49	6.6
		30	6.6	4.5	8.1	69.4	99.3	55.2	1.0	0.7	2	1.5	0.2	0.0	1.4	292	11.6
		40	6.4	4.5	8.2	73.5	126.1	50.4	0.5	0.1	33	0.2	0.2	0.0	0.0	430	11.3

C. Unraveling the importance of polyphenols for microbial carbon mineralization in rewetted riparian peatlands

Abstract

There have been widespread attempts to rewet peatlands in Europe and elsewhere in the world to restore their unique biodiversity as well as their important function as nutrient and carbon sinks. However, changes in hydrological regime and therefore oxygen availability likely alter the abundance of enzyme-inhibiting polyphenolic compounds, which have been suggested as a "latch" preventing large amounts of carbon from being released into the atmosphere by microbial mineralization. In recent years, a variety of factors have been identified that appear to weaken that latch including not only oxygen, but also pH. In minerotrophic fens, it is unknown if long-term peat mineralization during decades of drainage and intense agricultural use causes an enrichment or a decline of enzyme-inhibiting polyphenols. To address this, we collected peat samples and fresh roots of dominating plants (i.e., the peat parent material) from the upper 20 cm peat layer in 5 rewetted and 6 natural fens and quantified total phenolic content as well as hydrolysable and condensed tannins. Polyphenols from less decomposed peat and living roots served partly as an internal standard for polyphenol analysis and to run enzyme inhibition tests. As hypothesized, we found the polyphenol content in highly decomposed peat to be eight times lower than in less decomposed peat, while condensed tannin content was 50 times lower in highly degraded peat. In addition, plant tissue polyphenol contents differed strongly between peat-forming plant species, with the highest amount found in roots of *Carex appropinquata* at 450 mg g^{-1} dry mass, and lowest in *Sphagnum* spp. at 39 mg g^{-1} dry mass: a 10-fold difference. Despite large and clear differences in peat and porewater chemistry between natural and rewetted sites, enzyme activities determined with Fluorescein diacetate (FDA) hydrolysis and peat degradation were not significantly correlated, indicating no simple linear relationship between polyphenol content and microbial activity. Still, samples with low contents of polyphenols and condensed tannins showed the highest microbial activities as measured with FDA.

Introduction

Despite covering only 2–3 % of the land surface, peatlands store approximately one third of global soil carbon (C; Gorham, 1991; Jenkinson et al., 1991; Yu et al., 2010). Due to the anaerobic conditions in waterlogged peat, the rate of production of organic matter exceeds its decomposition (Pind et al., 1994; Williams and Yavitt, 2003; Freeman et al., 2004). Peatlands also play a significant role in water cycling and nutrient retention in river catchments (Verhoeven et al., 2006) and often support unique biodiversity (Zerbe et al., 2013). However, despite their importance from local to global scales, a significant loss in global peatland area continues (Joosten, 2010; IPCC, 2014) and attempts to restore these important ecosystems are widespread. In NE Germany, more than 30,000 hectares of drained minerotrophic (groundwater-fed) peatlands (i.e., fens) have been rewetted over the last 30 years to restore their ecological functions (Zak et al., 2018). Due to their extended drainage history, a re-establishment of their original state is not expected in a human life time perspective (Zak et al., 2018), but they may eventually return to a carbon neutral state (Joosten et al., 2015). The post-rewetting dynamics of carbon cycling in fens are, however, still poorly constrained.

The peatland carbon imbalance is regulated by the prevalence of polyphenolic compounds that inhibit microbial carbon mineralization and the enzymes that act upon them (Freeman et al., 2001). In ombrotrophic (rain-fed) peatlands (bogs), the enzyme phenol oxidase represents a key regulator for microbial carbon mineralization (Freeman et al., 2001, 2004). Phenol oxidase depends on molecular oxygen (O_2) and is highly active under drained conditions. This increased activity reduces the amount of polyphenolic compounds and limits their inhibiting effect on microbial decomposition. In contrast, when oxygen is depleted under waterlogged conditions, phenol oxidase is inactive, polyphenols accumulate, and microbial carbon mineralization becomes limited. This mechanism is so effective that it has been termed an "enzymic latch" on the peatland carbon store (Freeman et al., 2001). The "enzymic latch" theory is still discussed widely, with some suggesting greater complexity than previously assumed based on research in tropical peats (Hall et al., 2014) and temperate systems (Fenner and Freeman 2011; Brouns et al., 2016; Bonnett et al., 2017).

Despite its possible central role for organic matter decomposition in bogs, the "enzymic latch" has rarely been investigated in fens (but see Pinsonneault et al., 2016). This mechanism may be particularly relevant in previously drained fens, because prolonged drainage exposes the peat surface to oxygen and allows phenol oxidase to activate, essentially enhancing peat decomposition that may even continue under rewetted conditions, as long as polyphenol content is considerably reduced. In addition, drainage leads to warming of the soil. This may further weaken the “latch” since temperature seems to be an important control on phenol oxidase activity across peatland types (Pinsonneault et al., 2016). A biogeochemical legacy of draining is well-documented in several other aspects of fen carbon and nutrient dynamics. For example, a strong increase in methane emissions is typically measured in rich fens after rewetting (Hahn-Schöfl et al., 2011; Franz et al., 2016). Elevated concentrations of dissolved organic carbon, ammonium, and phosphate at levels of one to three orders higher compared to pristine systems have been measured in porewaters of the upper degraded peat layers of rewetted fens (Zak and Gelbrecht 2007), indicating potentially increased microbial turnover of organic matter in degraded topsoil of formerly drained fens. Laboratory incubations under stationary conditions have shown that microbially mediated redox processes are more intense in highly decomposed vs. less decomposed peat, partly explained by enhanced availability of nutrients and terminal electron acceptors (Zak and Gelbrecht 2007; Cabezas et al., 2012). On the contrary, there is evidence that the general availability of organic substrate within the decomposed peat layer is lower because it has already undergone mineralization over decades of drainage, and it is expected to be more recalcitrant due to the enrichment of polyphenols, in particular of lignin and tannin, that can inhibit enzymatic systems important for microbial carbon cycling (Bader et al., 2018).

Polyphenols are a large and diverse group of organic substances in which at least two hydroxyl (OH^-) groups are bound to aromatic ring structures. They are typical plant metabolites that are synthesized to support a variety of physiological functions such as morphology and energy metabolism (e.g., pigments), structure (e.g., lignin), and pathogen or predator resistance (e.g., flavonoids and tannins; Bravo 1998). Tannins are of particular ecological importance, as these high-molecular weight polyphenols (500–3,000 Da; Swain 1979) possess protein deactivation potential by forming insoluble protein complexes, and therefore affect many microbial processes that are enzyme-mediated (Bravo 1998). They are the fourth-most abundant class of biochemical compounds in terrestrial biomass after cellulose, hemicelluloses, and lignin, and are found in the bark, wood, leaves, fruits, and roots of a wide variety of vascular plants (Cowan 1999; Hernes and Hedges 2000; Mueller-Harvey 2001). Due to their multiple phenolic hydroxyl groups and high molecular weights, tannins, in contrast to low molecular weight phenolic acids such as gallic, vanillic, or ferulic acids, may limit litter decomposition more effectively in a number of different ways: (1) by complexing or deactivating microbial exoenzymes, (2) by complexing metals essential to enzyme activity, (3) by direct toxicity to microbes, (4) by themselves being resistant to decomposition, (5) by sequestering proteins in tannin-protein complexes that are resistant to decomposition and finally, (6) by coating other compounds, such as cellulose, and protecting them from microbial attack (Kraus et al., 2003). To unravel the importance of these single processes, it seems mandatory to complement bulk polyphenol analysis by further characterizing the biochemical properties of the polyphenol assemblage (Mole and Waterman 1987; Appel 1993; Nelson et al., 1997; Hättenschwiler and Vitousek 2000; Rautio et al., 2007). Analytically, tannins can be divided into two major groups: hydrolysable tannins and condensed tannins (Fig. C-1); the latter also termed proanthocyanidins (PAs). Hydrolysable tannins are composed of gallic acid or hexahydroxydiphenic acid esterified to a sugar moiety while condensed tannins are polymers of three ring flavan-3-ols joined with C-C bonds.

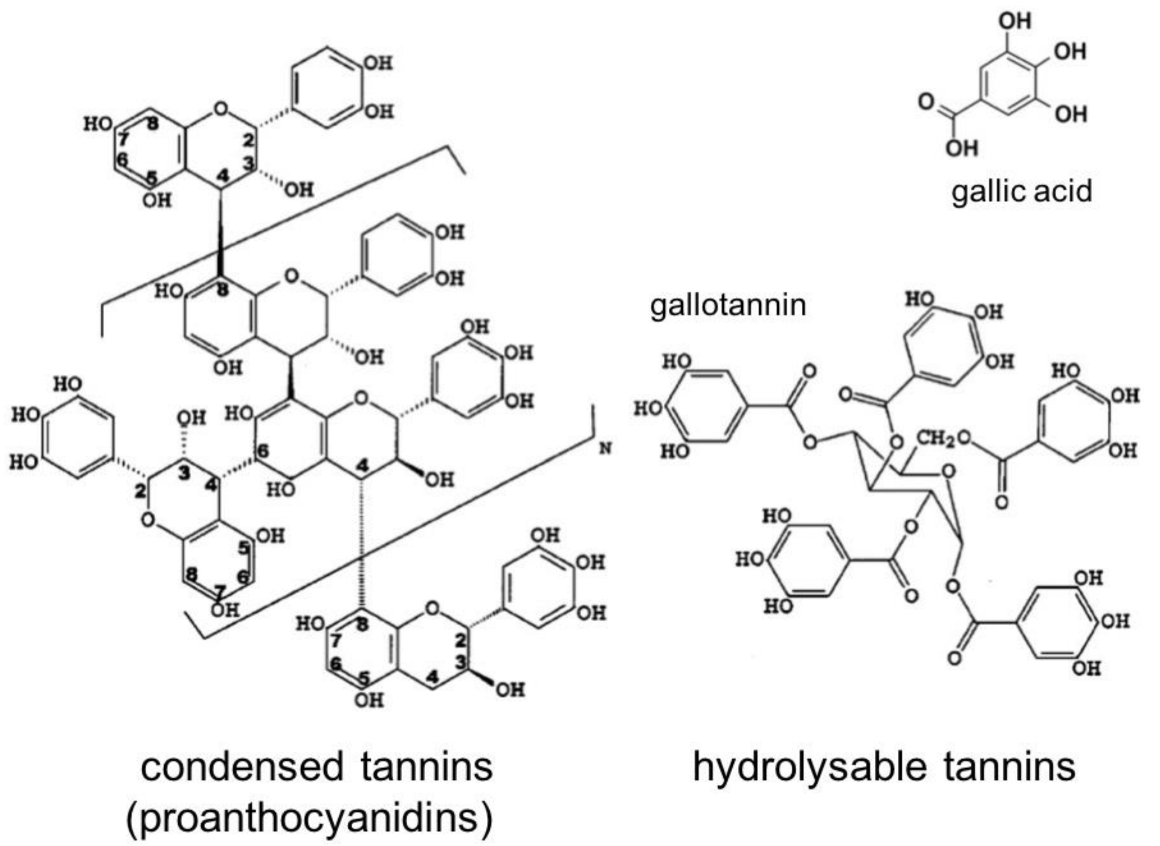


Figure C-1 – Structure of condensed and hydrolysable tannins (modified after Hernes and Hedges, 2000).

Due to these unique properties of polyphenols, we expect that their effect on carbon turnover should be similar to the "enzyme latch" described in bogs. We hypothesize that highly degraded peat of rewetted fens contains less enzyme-inhibiting polyphenols compared to less decomposed peat of pristine fens due to break up of polyphenols during the drainage phase. We analyzed fen peat substrates as well as peat-forming plant tissues from rewetted and pristine fens for representative polyphenol fractions—total phenol content, hydrolysable and condensed tannins—and for bulk microbial activity. Further, we tested microbial inhibition with PAs and characterized the peat samples chemically.

Methods

Study Sites and Sampling

The investigated fens are situated along freshwater sources in the postglacial landscape of northeast Germany and northwest Poland (Fig. C-2). The rewetted fens Hasenfelde, Sauwinkel, Menzlin, Zarnekow, and Jargelin have been subject to long-term drainage and were used mostly as grassland over several decades. At the time of sampling, their upper soil layer therefore consisted of highly decomposed peat until a depth of ~0.3 m, followed by less decomposed peat until a depth of up to 10 m (Zak and Gelbrecht, 2007). Three to Twelve years after rewetting, all sites were dominated by helophytes such as *Phragmites australis* and T*ypha latifolia* in permanent flooded areas, and by *Phalaris arundinacea* in areas experiencing wet-dry cycles (Zak et al., 2015).

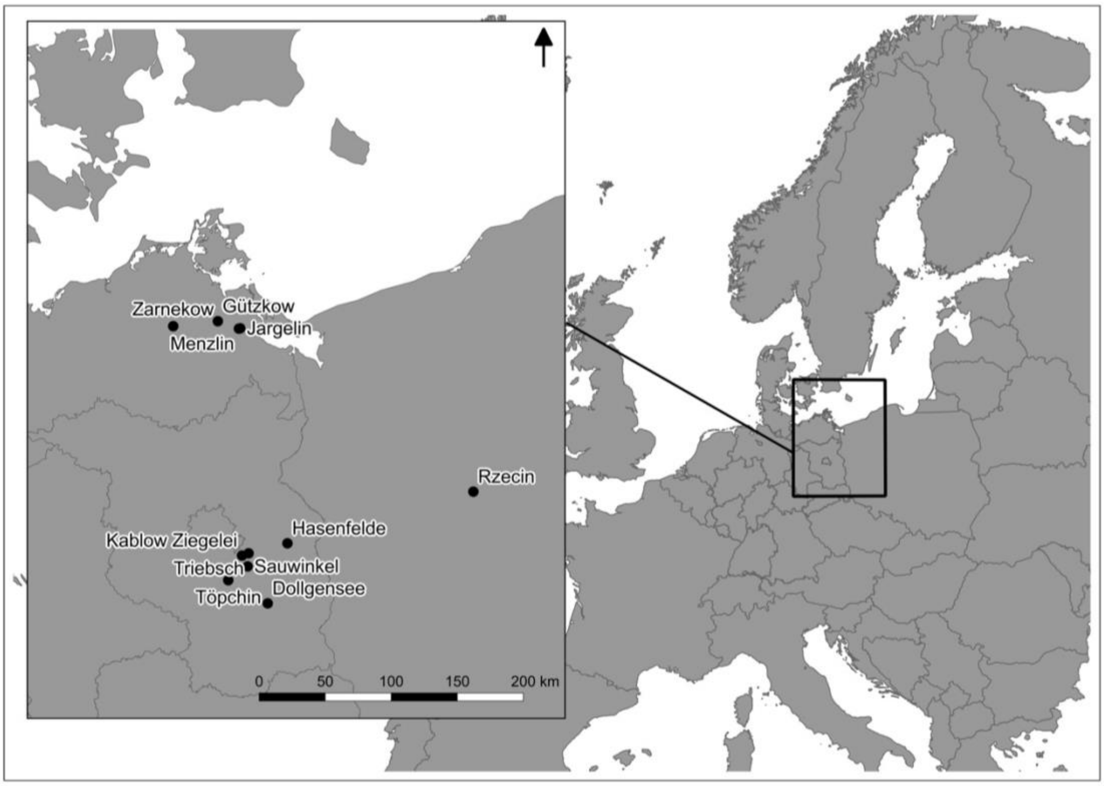


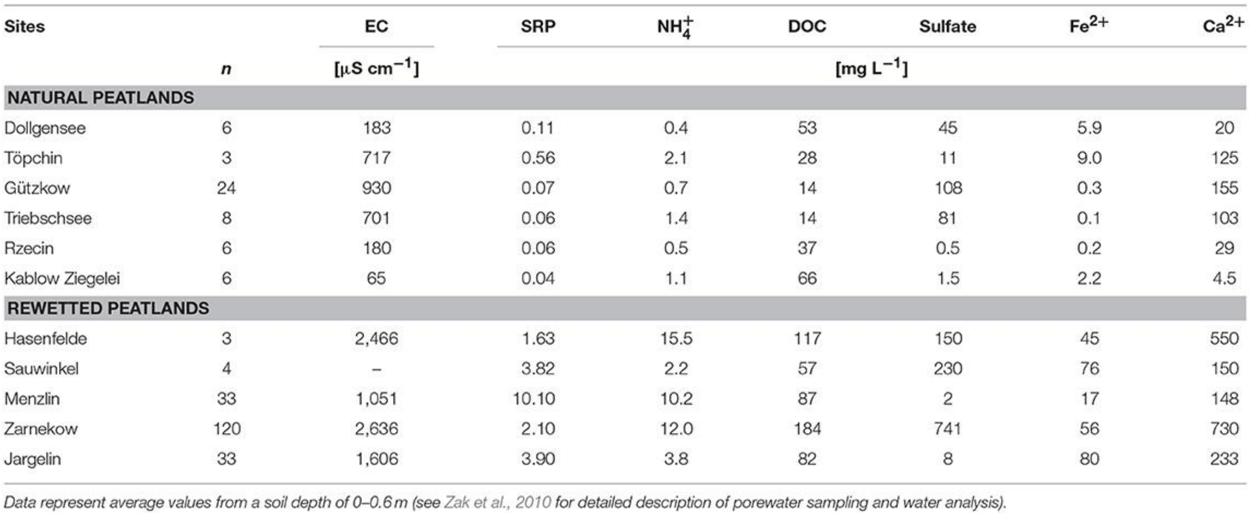
Figure C-2 – Location of sampled peatlands in NE Germany and NW Poland.

The hydrology of the sites Dollgensee, Töpchin, Gützkow, Triebschsee, and Rzecin was not or only slightly disturbed by drainage or similar measures in the past. In all areas the peat at the soil surface was only slightly decomposed, i.e., H1–4 according to the von Post humification scale

(Von Post 1922). All of these near to pristine peatlands can be classified as mesotrophic subneutral fens and are dominated by brown mosses (e.g., *Bryum pseudotriquetum*, *Campylium stellatum*, *Plagiomnium elatum*), and low sedges like *Carex hostiana*, *C. flacca*, and *C. panacea* (Zak et al., 2010). Furthermore, sparse stands of *Phragmites australis* occur in all the pristine sites, which represents the main peat-forming plant apart from *Carex* spp. and brown mosses. For the rewetted sites, the peat parent material was not recognizable any more due to the amorphous character of the soil, however deeper layers which were unaffected by water table drawdown indicate the same peat-parent material as in the undisturbed sites.

Peat samples were collected in triplicate from the top layers using a knife (ca. 10–20 cm depth) and transported to the lab within 1 day in an insulated cooler with ice, and analyzed for the characterization of total microbial activity immediately or frozen at −18°C for polyphenol analysis. Select data on porewater chemistry and peat characteristics are compiled in Tables C-1 and 2. A detailed description of conducted bulk peat analyses (total contents of phosphorus, carbon, nitrogen, iron, and organic matter), porewater sampling, and chemical analysis of dissolved matter concentrations (phosphate, ammonium, dissolved organic carbon, sulfate, iron, and calcium) can be found in Zak et al. (2010). Furthermore, plant samples (roots or moss litter) were taken from site Triebschsee (*Carex appropinquata*, brown mosses) and an additional natural poor fen, Kablow-Ziegelei (*Eriophorum angusitfolium*, *Vaccinium oxycoccos*, *Sphagnum* spp.) and treated analogously to the peat samples.

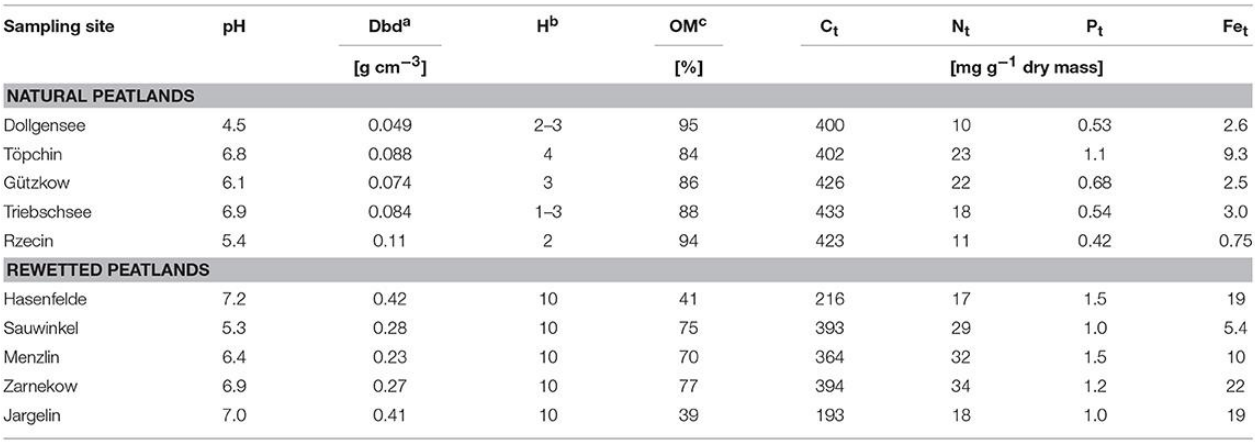
Table C-1 – Select data on porewater chemistry (EC—electrical conductivity, SRP—soluble reactive phosphorus).



Sites		EC	SRP	NH_4^+	DOC	Sulfate	Fe^{2+}	Ca^{2+}
	n	[µS cm^{-1}]	[mg L^{-1}]					
NATURAL PEATLANDS								
Dollgensee	6	183	0.11	0.4	53	45	5.9	20
Töpchin	3	717	0.56	2.1	28	11	9.0	125
Gützkow	24	930	0.07	0.7	14	108	0.3	155
Triebschsee	8	701	0.06	1.4	14	81	0.1	103
Rzecin	6	180	0.06	0.5	37	0.5	0.2	29
Kablow Ziegelei	6	65	0.04	1.1	66	1.5	2.2	4.5
REWETTED PEATLANDS								
Hasenfelde	3	2,466	1.63	15.5	117	150	45	550
Sauwinkel	4	–	3.82	2.2	57	230	76	150
Menzlin	33	1,051	10.10	10.2	87	2	17	148
Zarnekow	120	2,636	2.10	12.0	184	741	56	730
Jargelin	33	1,606	3.90	3.8	82	8	80	233

Data represent average values from a soil depth of 0–0.6 m (see Zak et al., 2010 for detailed description of porewater sampling and water analysis).

Table C-2 – Selected peat characteristics of sampling sites: (C_t, N_t, P_t, and Fe_t–total contents of carbon, nitrogen, phosphorus, and iron), dry bulk density (Dbd) calculated from the dry matter according to Scheffer and Blankenburg (1993), the degree of peat decomposition (H) according to von Post-scale (Von Post, 1922) and organic matter (OM) in % of dry matter determined by loss on ignition.



Sampling site	**pH**	**Dbd**[a]	**H**[b]	**OM**[c]	**C_t**	**N_t**	**P_t**	**Fe_t**
		[g cm^{-3}]		**[%]**		**[mg g^{-1} dry mass]**		
NATURAL PEATLANDS								
Dollgensee	4.5	0.049	2–3	95	400	10	0.53	2.6
Töpchin	6.8	0.088	4	84	402	23	1.1	9.3
Gützkow	6.1	0.074	3	86	426	22	0.68	2.5
Triebschsee	6.9	0.084	1–3	88	433	18	0.54	3.0
Rzecin	5.4	0.11	2	94	423	11	0.42	0.75
REWETTED PEATLANDS								
Hasenfelde	7.2	0.42	10	41	216	17	1.5	19
Sauwinkel	5.3	0.28	10	75	393	29	1.0	5.4
Menzlin	6.4	0.23	10	70	364	32	1.5	10
Zarnekow	6.9	0.27	10	77	394	34	1.2	22
Jargelin	7.0	0.41	10	39	193	18	1.0	19

Polyphenol Analysis

Sample Preparation and Extraction of Phenolic Substances

For the extraction of phenols, we followed the protocol of Hagerman (2002) taking into the account that phenols are not stable at light and higher temperature with slight adaptations. Thus, after removing coarse living plant roots, collected peat samples were freeze-dried, finely ground in an agate ball mill, and stored at 18°C in the dark. Phenolic substances were extracted with 10 mL 70 % aqueous acetone (v/v) three times sequentially from 0.2 g of the lyophilized peat material in screw cap plastic tubes. During extraction, tubes were suspended for 20 min in an ultrasonic water bath cooled to temperatures below 20°C. The used sonicator allowed samples to be maintained in the dark during the extraction period. The extracts were decanted after centrifugation at 10,600 g for 5 min (Universal 30F, Hettich, Tuttlingen, Germany) and combined in Erlenmeyer flasks on ice for further analysis. For the acid butanol assay and for purification of PAs from the extracts, 2 mg ascorbic acid L^{-1} were initially added to the 70 % aqueous acetone (v/v) to prevent oxidation of the extracted PAs. No ascorbic acid was added when extracts were used for the analysis of total phenolics since ascorbic acid strongly interferes with the color reaction by reducing the Folin-Ciocalteau reagent (Box, 1983; Georgé et al., 2005).

Isolation of Proanthocyanidins

PAs were obtained from peat samples of the five pristine sites following Giner-Chavez et al. (1997), where water-solved PAs are precipitated with trivalent ytterbium acetate, with slight modifications. The extraction procedure for obtaining of PAs was performed as described before with the exception that 1 g instead of 0.2 g of the lyophilized peat were extracted in order to yield higher amounts of tannins. The extraction was performed in six parallel runs to gain sufficient tannin material from each peat soil. After combination the extracts were washed three times with 50 ml n-hexane to remove lipids, and three times with 50 ml ethyl acetate to remove other remaining non-tannin components from the crude peat extract. Rotoevaporation was used in addition to separating funnels to remove acetone and traces of organic solvents yielding approximately 40 mL of an aqueous tannin solution. Ten ml 0.1 M ytterbium acetate ($Yb(C_2H_2O_2)_3 \cdot 4H_2O$) and 2 ml 0.5 M triethanolamine as buffer ($N(CH_2CH_2OH)_3$) were added to this solution and the extracts were stored in the dark for 20 h at 4°C to precipitate solved tannins. After centrifugation, the pellet was washed twice with 20 mL of 70 % acetone (v/v) and PAs were recovered from the formed complexes using cation exchange resin (Nyamambi et al., 2000). For this purpose, tannin ytterbium complexes were mixed with 2 g exchange resin (Amberlite IRP-64, Sigma-Aldrich, Schnelldorf, Germany) and with three times 40 mL 70 % acetone (v/v) sequentially in screw top glass tubes placed in a cooled sonicator for 20 min. Free PAs left in solution were then separated from the resin by centrifugation and filtration, from acetone by rotoevaporation and freeze dried to yield solid samples.

The PAs were used to determine their inhibitory activity on enzymes originating from peat, and as internal standards for colorimetric analyses. Analysis of UV spectra of the colored anthocyanidins formed during the acid butanol assay were conducted to verify the quality of isolated PAs and their suitability as internal standards (Fig. C-3).

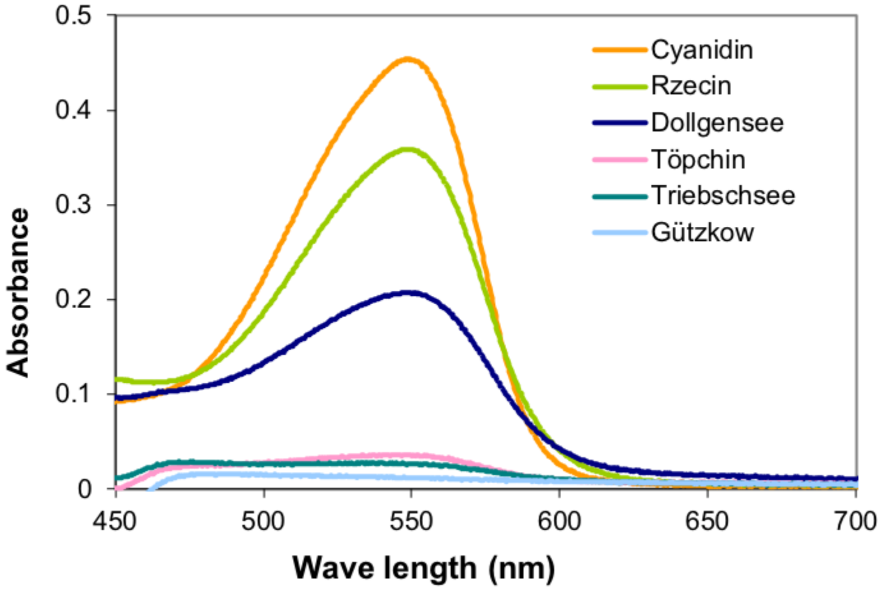


Figure C-3 – UV spectra of the colored anthocyanidins formed during the acid butanol assay were conducted to verify the quality of separated PAs and their suitability as internal standards for pristine peatlands.

Quantification of Total Phenolics

The simple, non-specific Folin-Ciocalteau assay quantifies total concentrations of phenolic hydroxyl groups in plant extracts (Box 1983). The method was slightly modified most notably by using only half of the given volumes and reducing reaction times to 30 min (Hilt et al., 2006). 0.3–0.5 mL aliquots of the combined extracts were increased to volumes of 5 ml with deionized water in 10 mL screw cap glass tubes to obtain absorbance below 0.5. After addition of 0.75 ml of sodium carbonate solution (75 g Na_2CO_3 L^{-1}) and 0.25 ml Folin-Ciocalteau reagent (Merck KGaA, Darmstadt, Germany), tubes were vortexed for 5 s. Absorbance was read at 750 nm (Photometer Spekol 221, Iskra Elektronik, Stuttgart, Germany) exactly 30 min after addition of the Folin-Ciocalteau reagent. The assay was calibrated with tannic acid (Fluka/Sigma-Aldrich, Munich, Germany) and tannins isolated from peat material. Analyses for polyphenols were carried out on three technical replicates per field sample. The same holds for all steps regarding the general chemical characterization of the field samples.

Quantification of Hydrolysable Tannins

Hydrolysable tannins were measured after saponisation and extraction after Luthria and Pastor-Corrales (2006) modified or peat soils. Specifically, 0.2 g of the lyophilized and grounded peat was digested by adding 2.5 mL 2 M NaOH containing 10 M EDTA and 1 % ascorbic acid in an ultrasonic water bath at 45°C. The extraction was conducted with 4 mL ethyl acetate after acidification with 7.2 mL 2 M HCl. After shaking for 30 min the samples were centrifuged at 10,600 g for 5 min. The extraction was repeated one time and combined supernatants were dried by rotoevaporation under nitrogen atmosphere. Capturing was done with 300 µL 0.1 % formic acid. Analyses were conducted using High-Performance Liquid Chromatography (Gynkotek) with UV detection at 280 nm. Separation was achieved using the column Phenomex Prodigy PH3 5 µ (250 × 4.6 mm) at constant flow mode and temperature (1.9 mL min^{-1}, 25°C). The mobile phase was a constant mixture of methanol/0.1 % HCOOH (80%/20%, v/v). For calibration, gallic acid was used (Fluka/Sigma-Aldrich, Munich, Germany).

Quantification of Condensed Tannins

Condensed tannins or PAs were, respectively, measured with the acid butanol method (Porter et al., 1986), by adding 6 mL acid butanol reagent (nbutanol/concentrated HCl, 95:5 by volume) and 0.2 mL iron reagent (2 % ferric ammonium sulfate dodecahydrate salt, $FeNH_4(SO_2)_4 \cdot 12H_2O$, in 2 M HCl) to 1 ml extract solution. Samples were then vortexed for 5 s and placed for 50 min in a water bath at 95°C. After cooling the samples to room temperature, absorbance was measured at

550 nm (Photometer Spekol 221, Iskra Elektronik, Stuttgart, Germany). Since samples from different peat soils were already colored to different extents before heating, absorbance was also read before the production of colored anthocyanidins and subtracted from the values measured after heating. This approach led to a conservative estimate of tannin concentrations, since some chromophores might have been destroyed upon heating (Hagerman 2002). Analyses of UV spectra (200–700 nm) were conducted to confirm the subtraction of background color. The acid butanol assay was calibrated with cyanidin chloride (Carl Roth, Karlsruhe, Germany) and the tannins isolated from peat material.

Microbial Activity in Peat Soils

Fluorescein diacetate (FDA) hydrolysis was performed to measure the enzyme activity of microbes according to Schnürer and Rosswall (1982) and optimized for peat analysis. Always, ca. 0.6 g (± 0.1 g) fresh peat were directly weighed into screw cap glass tubes out of ca. 15 g fresh bulk peat sample. The samples were mixed with 4 mL potassium phosphate buffer (pH 7.6; 8.7 g K_2HPO_4 and 1.3 g KH_2PO_4 in 1 L distilled water), 1 mL distilled water, and 0.3 mL FDA solution (1 mg FDA in 1 mL acetone). Peat slurries were shaken then for 25 min on a rotary shaker and centrifuged at 4200 g for 5 min (Heraeus Labfuge 400, DJB Labcare, Buckinghamshire, England). 0.5 mL of the supernatant was diluted with 1.5 mL potassium phosphate buffer solution before measurement at 490 nm on a spectrophotometer (Photometer Spekol 221, Iskra Elektronik, Stuttgart, Germany). Blanks were measured without the addition of FDA to correct for background absorbance and concentrations of released fluorescein were determined by calibration with a fluorescein sodium salt solution ($C_{20}H_{10}Na_2O_5$, Sigma Aldrich, Schnelldorf, Germany). Hydrolysis rates of FDA were expressed as μg fluorescein h^{-1} mg^{-1} oven dry (105°C) peat. Dry matter of aliquots withdrawn from the slurry and of weighed peat soils from pristine sites were measured for each analytical run.

Inhibition of Microbial Activity with Proanthocyanidins

To evaluate the inhibitory activity of PAs, the FDA hydrolysis assay was performed as described above, adding PAs dissolved in 1 mL distilled water to a 4 mL slurry just before starting the hydrolysis reaction with FDA solution. Absorbance of formed fluorescein at 490 nm was related to a reference sample that did not contain any tannin and results were expressed in percentage inhibition. PAs from the five pristine sites as well as tannic acid (Fluka/Sigma-Aldrich, Munich, Germany) as model substance were added to yield final concentrations of 100 mg L^{-1} in the test tubes. This concentration corresponded to a tannin/peat ratio of ~1:140. Additionally, PAs from

Rzecin and tannic acid were added to yield concentration related inhibition with concentrations between 0–150 mg L^{-1} and 0–400 mg L^{-1}, respectively. All inhibition tests were conducted with highly decomposed peat material from a long-term experiment containing peat from Zarnekow (Zak and Gelbrecht 2007). Analyses of microbial activities and inhibition of microbial activity was carried out on four to five technical replicates of each field sample.

Statistical Analysis

Statistical tests were performed using SPSS 14.0 for windows (SPSS Inc., 2005) and R (R Core Team, 2018). On the one hand, we simply compared the variables introduced above across two peatland types—degraded and near-natural—treating the samples from the different sites as replicates among peatland types. We analyzed whether there were significant differences between samples from these two peatland types with respect to the inhibitory activity of proanthocyanidins on enzyme-activity in samples, the amounts of phenolics as well as of hydrolysable and condensed tannins, and microbial activity using either one-way ANOVA or alternatively the parameter free Kruskal–Wallis test when conditions for the ANOVA were not satisfied. Significance was tested against an α level of 0.05.

On the other hand, we investigated the relationships between the total concentrations of phenolic hydroxyl groups (A750) and condensed tannins (A550), the estimated total microbial activity according to the FDA analysis (FDA), and the multivariate characteristics of the peat (OM, C:N, C, N, P, Fe:P, P, bulk density, von Post scale) with NMDS (non-metric multi-dimensional scaling) ordination. NMDS allows for the exploration of multivariate data in low dimensional space. The NMDS based on the characteristics of the peat was performed with function metaMDS() of package vegan (Oksanen et al., 2017) for R with default settings. Sub-sequentially we fitted the above stated variables to the ordination result using the function envfit() of package vegan. This approach is closely related to the concept of multiple regression and allows to investigate the possible relationship of a number of predictors on the multivariate configuration of the peat samples defined by all their relevant characterizing variables. The significance of relationships was determined with the build in permutation test of envfit() (999 runs). Variables with $p < 0.05$ were deemed significantly related to the ordination configuration.

To test whether microbial activity was determined by inhibitory compounds (A750, A550) and/or some of the other peat characterizing variables we constructed a multiple linear regression with estimated total microbial activity (FDA) as response and all variables that were identified as significant in the NMDS as drivers. The primary model was then further simplified using the function step() in R that runs an iterative approach of combined forward and backward selection, using the lowest AIC as selection criterion for deciding on the final regression equation.

Results

Proanthocyanidins

Extraction of PAs from the different slightly decomposed peat soils yielded <1 % of initial peat material on a dry matter basis. Although precipitation of tannins with trivalent ytterbium was not used for gravimetric quantification, it was a good indicator of tannin concentration in washed extract solutions. Extracts from peat of the pristine sites Rzecin and Dollgensee almost immediately showed precipitation of tannin-ytterbium complexes whereas peat extracts from Gützkow precipitated small amounts only perceptible after centrifugation. Calibrations of the acid butanol and the Folin-Ciocalteau assay with separated tannins are presented in Fig. C-4. Separated tannins from Gützkow, Triebschsee and Töpchin were characterized by particularly low absorbance values (<0.035 at 550 nm) in the acid butanol assay. Analysis of UV spectra showed that, in the case of tannins isolated from Gützkow peat, no absorbance maximum could be detected at 550 nm (Fig. C-3) although tannin concentrations were as high as 0.61 mg mL^{-1} (Fig. C-4). In the cases of Triebschsee and Töpchin, absorbance maxima could be identified but were not significantly different from background absorbance. Therefore, only calibrations for peat from the sites Rzecin and Dollgensee were used as internal standards for the quantification of PAs. All tannins, however, showed relevant concentrations of total phenolics during the Folin-Ciocalteau assay. Comparison of internal standards and the commercially available standards cyanidin chloride and tannic acid illustrates the advantage of internal standards. In any case, quantification with cyanidin or tannic acid would lead to a considerable underestimation (80–97 %) of phenolic concentrations in peat soils (Table C-3). Unfortunately, the purification of internal standards is impractical when peat samples contain only small amounts of tannins. Consequently, as no adequate internal standards could be separated for each peat soil, especially not for highly decomposed peat, further comparisons of concentrations were conducted with values expressed in absorbance units (AU) per dry mass (AU750/AU550 g^{-1} DM). However, to enable comparison with other studies, AU values were translated to commonly used tannic acids equivalents and cyanidin equivalents (TAE/CAE g^{-1} DM) as summarized in Table C-3.

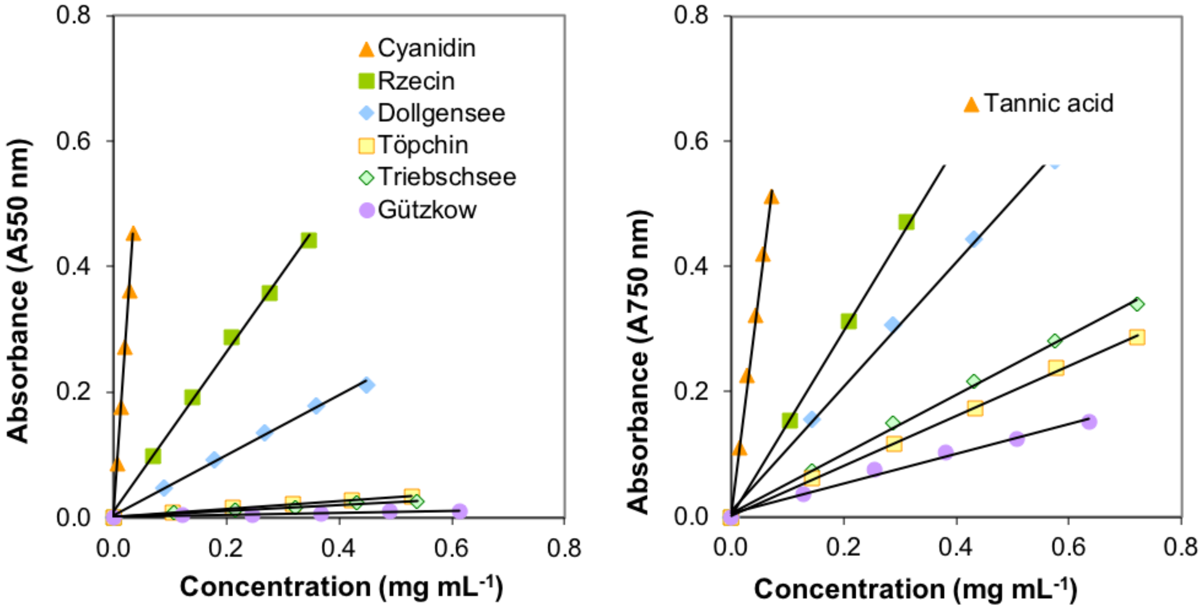
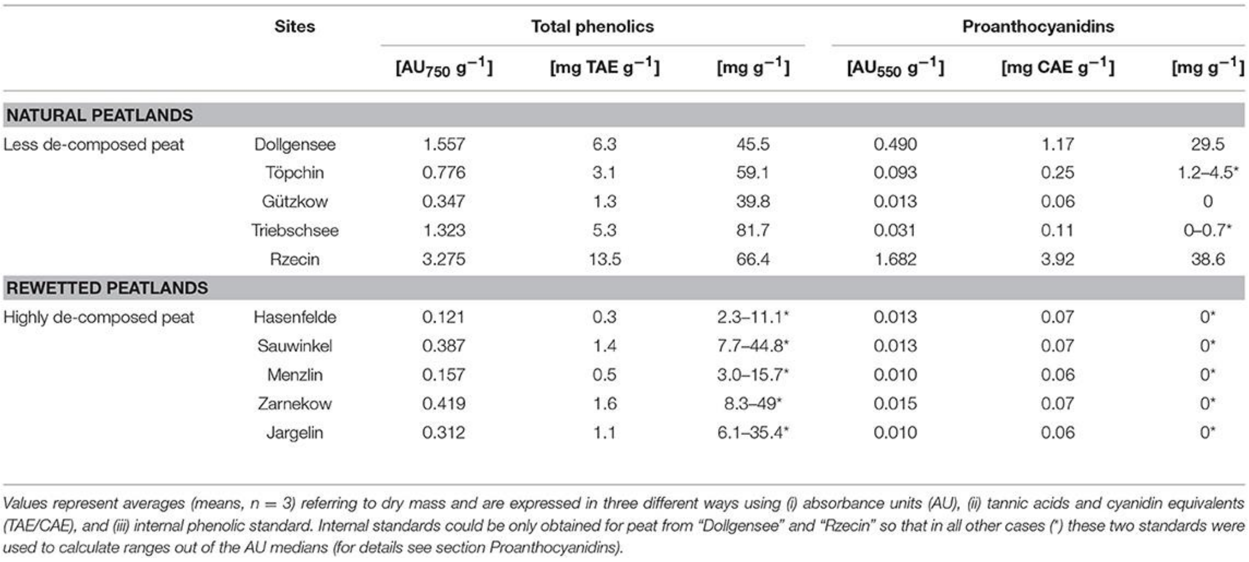


Figure C-4 – Calibrations of the acid butanol (left image) and the Folin-Ciocalteau assay (right image) with isolated tannins from less decomposed peat of pristine peatlands.

Table C-3 – Total phenolics and proanthocyanidins in differently decomposed peat soils referring to dry mass.



	Sites	Total phenolics			Proanthocyanidins		
		[AU$_{750}$ g^{-1}]	[mg TAE g^{-1}]	[mg g^{-1}]	[AU$_{550}$ g^{-1}]	[mg CAE g^{-1}]	[mg g^{-1}]
NATURAL PEATLANDS							
Less de-composed peat	Dollgensee	1.557	6.3	45.5	0.490	1.17	29.5
	Töpchin	0.776	3.1	59.1	0.093	0.25	1.2–4.5*
	Gützkow	0.347	1.3	39.8	0.013	0.06	0
	Triebschsee	1.323	5.3	81.7	0.031	0.11	0–0.7*
	Rzecin	3.275	13.5	66.4	1.682	3.92	38.6
REWETTED PEATLANDS							
Highly de-composed peat	Hasenfelde	0.121	0.3	2.3–11.1*	0.013	0.07	0*
	Sauwinkel	0.387	1.4	7.7–44.8*	0.013	0.07	0*
	Menzlin	0.157	0.5	3.0–15.7*	0.010	0.06	0*
	Zarnekow	0.419	1.6	8.3–49*	0.015	0.07	0*
	Jargelin	0.312	1.1	6.1–35.4*	0.010	0.06	0*

Values represent averages (means, n = 3) referring to dry mass and are expressed in three different ways using (i) absorbance units (AU), (ii) tannic acids and cyanidin equivalents (TAE/CAE), and (iii) internal phenolic standard. Internal standards could be only obtained for peat from "Dollgensee" and "Rzecin" so that in all other cases (*) these two standards were used to calculate ranges out of the AU medians (for details see section Proanthocyanidins).

Polyphenol Contents

Absorbance values of extractable total phenolics and PAs ranged between 0.121–3.275 AU750 g^{-1} DM and 0.01–1.682 AU550 g^{-1} DM, respectively (Table C-3). Hydrolysable tannins were not detectable in any of the peat samples. Both total phenolics and PAs were significantly higher in peat soils from pristine sites than in peat soils from degraded sites, with a strong correlation between both factors (Spearman's R 0.92). Notably, peat from Gützkow did not show higher values in comparison to the degraded sites and peat from Triebschsee had comparatively high concentration of total phenolics not reflected in high PA concentrations. The two sites Rzecin and Dollgensee stood out by their 139- and 40-fold higher concentrations of extractable PAs compared to degraded sites. Calibration of the acid Butanol and Folin-Ciocalteau assay with the same internal standard allowed an estimate of the size of the PA fraction in terms of total phenolics for peat from Rzecin and Dollgensee. Here, proanthocyanidins accounted for 58 and 65 % of total phenolics, respectively, with total phenolics representing 6 % of peat soil organic matter on an average.

Microbial Activity and Inhibition

The microbial activity of studied peat soils measured as hydrolysis rate of FDA ranged between 0.4 and 1.57 µg fluorescein h^{-1} mg^{-1} DM (Fig. C-5). The production of fluorescein was not significantly lower in the slightly decomposed peat soils, although Dollgensee and Triebschsee clearly had the lowest rates of FDA hydrolysis. However, peat from Gützkow showed very high activities for an only slightly decomposed peat soil, with presumably low microbial activities, and had the second highest activity after Menzlin.

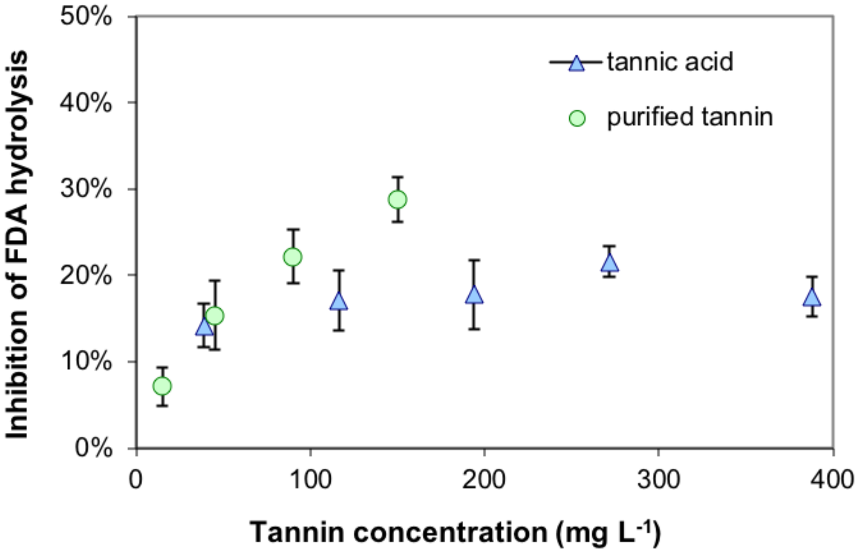


Figure C-5 – Microbial activity of studied peat soils measured as hydrolysis rate of fluorescein diacetate (FDA) for pristine peatlands with less decomposed peat (white bars) and rewetted peatlands with highly decomposed peat (gray bars).

All extracted tannins from pristine sites generated a significant inhibition of FDA hydrolysis when added to highly decomposed Zarnekow peat, with the exception of tannins from Gützkow peat (Fig. C-6). Significant inhibitions ranged between 12.3 and 17.9 % and were achieved without any preliminary incubation times of tannins mixed with peat material. These values were lower compared to the inhibition produced by the addition of tannic acid as model substance (30.1 %).

Suppression of FDA hydrolysis with tannins was poorly reproducible between runs, most probably because of the great heterogeneity of the analyzed peat material. We attribute varying inhibition to the fact that inhibition tests with 100 mg tannins L^{-1} were below saturation point (Fig. C-7).

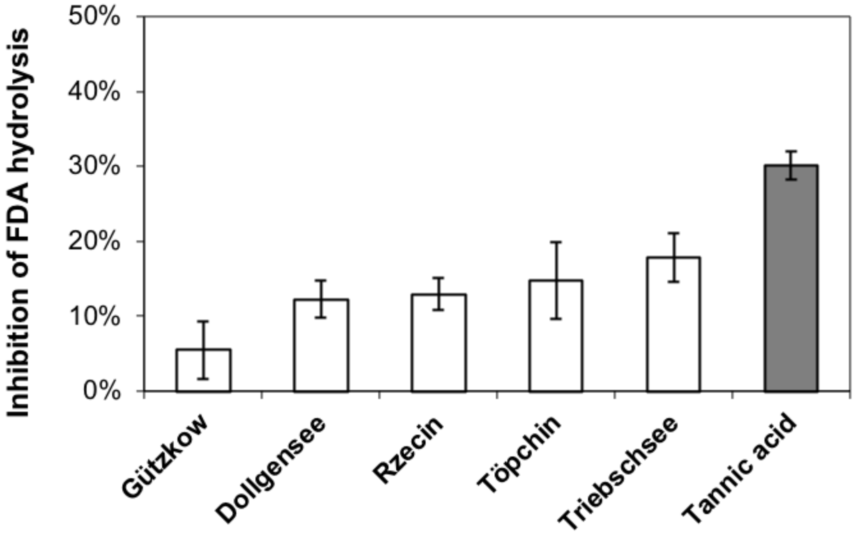


Figure C-6 – Inhibition of fluorescein diacetate (FDA) hydrolysis in highly decomposed peat from rewetted peatland "Zarnekow" using isolated tannins from less decomposed peat of pristine peatlands (white bars) and commercial tannic acid standard (gray bar).

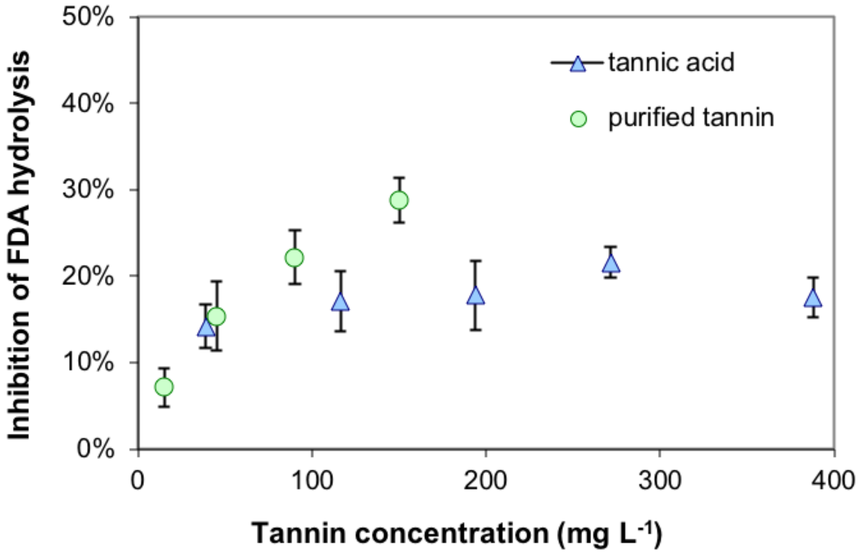


Figure C-7 – Suppression of fluorescein diacetate (FDA) hydrolysis with increasing concentrations of tannins at the example of highly decomposed peat from rewetted peatland "Zarnekow" using commercial tannic acid and a isolated tannin of less decomposed peat from pristine peatland "Rzecin".

Relationships Between Variables

The peat from the pristine and the altered sites are clearly separated in the NMDS plot (Fig. C-8) while the generalizing characteristic of the pH value (size of bubbles in Fig. C-8) doesn't seem to play an important role. The iron contents in the sites, mainly the Fe^{2+} concentrations in the porewater, have a strong influence on the ordination pattern and are associated with relatively low contents of organic matter (OM) in the peat. A similar negative relationship appears for the microbial activity (as measured with FDA) and the two variables representing total phenolic compounds (A750) and condensed tannins (A550) that were fitted post-hoc to the ordination and point at opposite directions (Fig. C-8). Interestingly these variables don't follow the pristine—rewetted gradient but are almost orthogonal to the latter. Only A750 is significantly related to the ordination configuration. While C and N in the peat show a similar direction like the organic matter, the CN ratio increases with the content of polyphenolics. Soluble reactive phosphorus increases in the opposite direction and thus relates well to increased microbial activity (Fig. C-8). We tested all variables for relationships to the microbial activity. Only three showed significant relation according to singular linear models: N content, A550, and A750 after omitting Rzecin which had a much lower Fe content (with high coefficient of variation) and a much higher total phenolic content (A750) than all other samples. Only total polyphenol content (A750) had a strong negative linear relationship with microbial activity ($R^2 = 0.83$, $p < 0.001$; Fig. C-9). This result was confirmed by the multiple regression approach. While N content, A750 and A550 (including a possible interaction between the latter) entered the analysis, the final model contained only total polyphenol content as a driver and was, thus, essentially the model depicted in Fig. C-9.

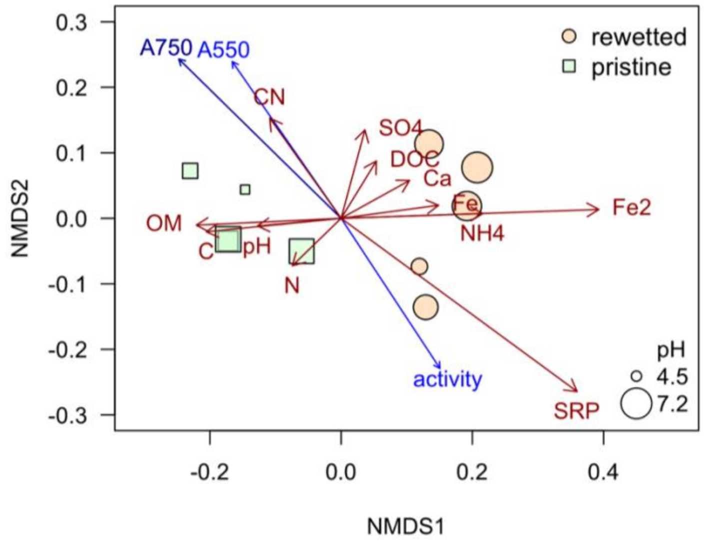


Figure C-8 – NMDS plot based on the chemical characteristics of the peat and porewater analyses. The NMDS was run with defaults using function metaMDS of package vegan in R. Final stress was 0.059. Red arrows represent the included variables with vector lengths ≥0.1. Blue arrows are the post-hoc fitted *variables on phenolic compounds and FDA, dark blue represents a variable that is significantly related to the ordination configuration with p < 0.05.*

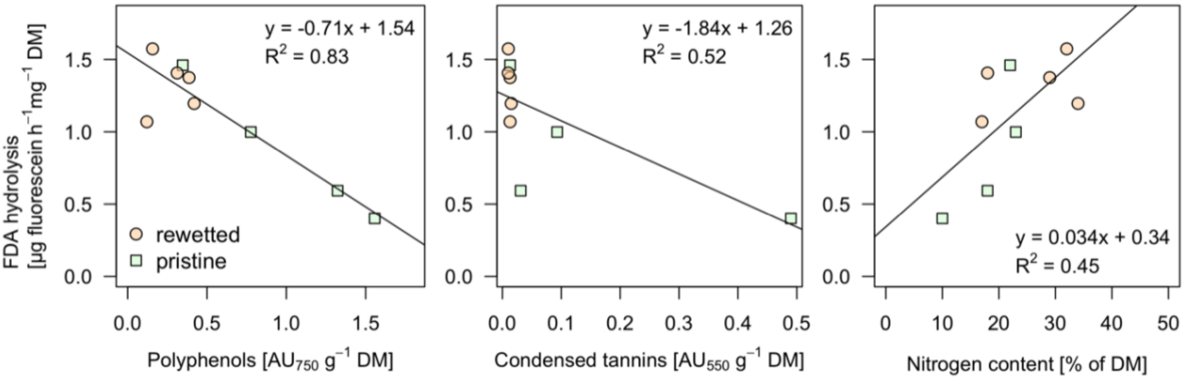
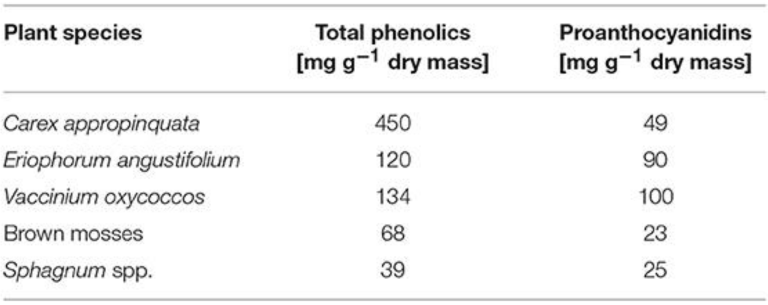


Figure C-9 – Significant linear relationships between microbial activity and other measured variables. The microbial activity was determined as enzyme activity using fluorescein diacetate (FDA) hydrolysis. Values are given on dry mass (DM) basis. The complete variable set (peat chemistry, pore water chemistry, and potentially inhibiting substances) were tested. Only relationships with linear models significant with p < 0.05 are displayed.

Discussion

Supporting our initial hypothesis, we found that highly degraded peat contained eight times lower levels of total polyphenols and 50 times lower levels of condensed tannins than less decomposed peat. Thus, draining and subsequent peat degradation effectively removes major parts of the tannin stock of fens by former mentioned oxidation processes and eliminates their potential function as an "enzymic latch" on the fen carbon store. The total phenolic contents of less decomposed peat from the pristine fens in this study (Table C-3) corresponded well with concentrations of less decomposed *Sphagnum* peat (ca. 4–8 mg TAE g^{-1} DM) from different European peatlands (Dorrepaal et al., 2005; Bragazza and Freeman 2007). Since peat-parent material in our study was mostly polyphenol-rich *Carex* spp. (Table C-4) higher contents could be expected but certain *Carex* species might differ strongly regarding polyphenol contents/composition and some part of polyphenols might be decomposed during peat formation (see below). The concentrations in peat porewater were much higher and ranged up to 100 mg L^{-1}. To put this into perspective, inhibitory effects on hydrolase activity were already recorded under phenolic contents between 2 and 5 mg L^{-1} for a Welsh peatland (Freeman et al., 2004). Soil pore water was not analyzed for polyphenols in this study, however according to the amount of water-extractable polyphenols of 0.7 mg TAE g^{-1} DM and a dry bulk density of 100 g L^{-1}, as determined for peat from Dollgensee (results not shown) inhibition-relevant porewater concentration of about 70 mg TAE L^{-1} can be expected.

Table C-4 – Average contents of total phenolics and proanthocyanidins (median, n = 3) in roots from selected peat-forming vascular plant and from moss litter (for plant sampling sites see section Study sites and sampling).



Plant species	Total phenolics [mg g^{-1} dry mass]	Proanthocyanidins [mg g^{-1} dry mass]
Carex appropinquata	450	49
Eriophorum angustifolium	120	90
Vaccinium oxycoccos	134	100
Brown mosses	68	23
Sphagnum spp.	39	25

Naturally, the complex nature of polyphenols with potentially more than 8,000 individual molecule structures cannot be reflected in the analysis of total phenolics. Nevertheless, this unspecific proxy has shown some good correlation with decomposition patterns in peatland studies (Dorrepaal et al., 2005; Bragazza and Freeman, 2007; Bragazza et al., 2007) and has proven to be a suitable predictor for tannin inhibition of microbial growth of ruminal bacteria (Nelson et al., 1997). Individual processes such as litter decay, however, may depend much more on the type of polyphenol, rather than just on the total content. Despite their large diversity, their functioning with regards to metabolic processes can be classified as "ineffective," "inhibiting," or "promoting" (e.g. Bonnett et al., 2017). The latter refers inter alia to their ability to mediate redox-processes in soils as described for the quinone already in the beginning of the Twentieth century (Erdtman 1933a, b). Likewise, the inhibitory properties of phenolics are well-known even for more than 100 years for *Sphagnum*-derived phenolic acids as earlier reviewed by Verhoeven and Liefveld (1997). The suppression of microbial activity in *Sphagnum* peat was partially attributed to a tanning-like process involving 5-keto-D-mannurionic acid, associated with contributing to the famous preservation property of *Sphagnum* bogs (Vanbreemen 1995). In vascular plant-dominated minerotrophic peatlands, tannins e.g. derived from *Eriophorum* spp. (Williams et al., 2000), could play an equally important role in the inhibition of microbial activity. Accordingly, it seems advantageous to obtain more detailed characterization of the biochemically diverse tannin fraction than to measure total phenolic contents alone.

The better solubility of hydrolysable tannins in water and their distinct complexing behavior, which depends on prevailing pH and redox conditions (Martin and Martin, 1983; Appel, 1993), promote them as potent enzyme inhibitors. On the other hand, their stability even in the form of tannin-protein-complexes may be too low to persist in the long term (Bravo 1998; Fierer et al., 2001). According to the absence of hydrolysable tannins in all tested peat soils, the inhibiting role of tannins must be solely attributed to the group of condensed tannins. Unfortunately, there is no reference for PA concentrations in peat, but some information is available for plant samples. For example, for conifers, the proportion was higher than 50 % of total phenolic content, similar to peat samples from the natural sites Dollgensee and Rzecin. While our data analysis shows that isolated PAs from less decomposed peat of natural peatlands inhibited the activity of hydrolases (Fig. C-7), enzyme activities did not differ significantly between different degraded peat samples (Fig. C-5). Still, at least when correcting for an obvious outlier we find a strong linear relationship between total polyphenol content and microbial activity (Fig. C-9), supporting the validity of the "enzymic latch" for fen peat. However, the linear relationship between the content of condensed tannins and microbial activity hinges on one data point only and the data rather suggest a threshold below which microbial activity can go up.

We assume that enzyme activity levels and decomposition of organic matter are not only related to the presence or absence of polyphenols, but are a concert of multiple factors and processes which is also represented in the direction of the dichotomy between microbial activity and the inhibitory compounds in the NMDS plot which does not follow the gradient from pristine to altered sites that is otherwise well-represented along the first NMDS axis. Yet another limiting factor is the availability of resources (C and other nutrients) for decomposing organisms (Yavitt et al., 2005). Litter decomposition thus depends on substrate quality, e.g., C:N ratio, and lignin content. The bulk parameter C:N ratio scales well with the amount of potentially inhibitory substances in the NMDS. Polymers such as cellulose and hemicelluloses are readily degraded by microbial exoenzyme systems into energy-yielding dimers and monomers. Lignin or lignin-like phenolic polymers as found in *Sphagnum* are more recalcitrant in comparison.

Plant litter with high nutrient concentration generally decomposes relatively fast (Couteaux et al., 1995; Laiho 2006). In *Sphagnum* litter, Bragazza et al. (2007) found the polyphenol/nutrient ratio to be one of the primary parameters controlling decomposition. Unlike in pristine bog peatlands, the decomposition of organic matter in the degraded and rewetted fens is strongly controlled by the biogeochemical regime including the elevated availability of electron acceptors like ferric iron and sulfate, enhanced nutrient availability, and circum-neutral pH (Zak and Gelbrecht 2007; Brouns et al., 2014). Respiration measurements with different organic substrates from rewetted peatlands suggest that degraded peat without any fresh plant-derived material is relatively inert in terms of decomposition, whereas significant anaerobic CO_2 and CH_4 production in peat only occurs when enough labile organic matter is available (e.g., from remaining roots or root exudates; Hahn-Schöfl et al., 2011). The role of polyphenols for the decomposition of fresh aboveground litter and from wetland plants was tested by anaerobic incubation experiments showing that the availability of nutrients rather than polyphenol contents were significant factors (Zak et al., 2015). The polyphenol concentrations of tested plant litter samples ranged between 14 and 68 mg g^{-1} DM and were, thus, at the lower level of root and moss litter from this study (39–450 mg g^{-1} DM, Table C-3). Unfortunately, the proportion of tannins was not quantified in this work, however it was interesting to note that in non-peat forming plants (*Phalaris arundinacea*, *Ceratophyllum demersum*, *Typha latifolia*) the content of polyphenols was reduced between 50 and 95 %, while in the peat forming plants (*Phragmites australis*, *Carex riparia*) the reduction was much lower (<20 % after 150 days).

At time of peat sampling, non-peat forming plants still dominated the studied rewetted sites. After 10 to 20 years of rewetting, however, a distinct succession toward peat-forming reed communities is evident, while the re-colonization with brown mosses and low sedges characterized by higher polyphenol contents seems to be retarded over several decades, depending on the drainage and land use history (Zerbe et al., 2013; Zak et al., 2015) as observed in other rewetting projects (Koch et al., 2017). Also, if peat forming plants are re-established the quality and quantity of polyphenols in plant tissues might differ substantially from their natural counterparts as long as nutrient levels are higher as under pre-drained conditions. Low nutrient levels are known for promoting the production of secondary metabolites inter alia to protect the plants against herbivory or pathogens (Verhoeven and Liefveld 1997). All things considered, it remains uncertain about how long it would take for enzyme-inhibiting polyphenols in soils to reach concentrations that would have a significant impact on microbial metabolism. For such an assessment it might also be considered that phenol oxidases could be activated under oxygen-free conditions in case of elevated concentrations of other electron acceptors, such as sulfate and oxidized but soluble or less crystalline ferric iron (Van Bodegom et al., 2005; Fenner and Freeman 2011). Thus, elevated sulfate concentrations in soil water of the pristine sites Gützkow and Triebschsee (>80 mg L^{-1}, Table C-1) might be responsible for the comparatively low polyphenol contents in peat from such sites. Eventually, there might be other anaerobic decomposition pathways depending on certain abiotic factors and/or presence of fungal and bacterial communities (e.g., Fuchs et al., 2011) which needs detailed consideration in order to better understand different patterns of organic matter decomposition in peatlands.

Conclusions

The drainage of fens and their use as grassland over approximately three decades caused a significant loss of condensed tannins. Accordingly, organic matter decomposition may continue at about the same pace as before the rewetting because of the (almost) absence of the "latch" provided by the phenols. Despite their presumed role as enzyme-inhibiting substances, hydrolysable tannins may play an insignificant role in decomposition processes since they do not accumulate in the peat in contrast to condensed tannins. Their capacity to reduce the microbial enzyme activity was clearly confirmed in assays using tannins from less decomposed peat of pristine fens. However, the substantial differences in polyphenol contents, in particular for condensed tannins, did not correspond with significant differences in hydrolytic enzyme activities in highly decomposed peat compared to less decomposed peat. Future research must clarify if and how long it may take after rewetting for polyphenol content and composition to return to those of pristine fens, and whether the repression of hydrolase enzymes eventually resumes, as this will be important for the long-term carbon accumulation. According to the high complexity of possible metabolic processes and driving factors both under aerobic and anaerobic conditions the measurement of single parameters like redox-conditions, nutrient availability or pH will be not sufficient to make a reasonable estimate of carbon turnover in rewetted peatlands. However, it can be presumed that the re-establishment of peat-forming *Carex* species or brown mosses characterized by high tannin contents could represent the switch for the "enzymic latch" mechanism. Another important precondition might be a significant reduction of electron acceptor availability and nutrient levels (Brouns et al., 2016). Altogether, there is evidence that polyphenol elimination by fen drainage must be ameliorated before rehabilitation of rewetted peatlands in the context of carbon cycling can be considered a success in decades to come. The removal of the degraded peat layer can be a suitable method to re-establish low-nutrient conditions and facilitate the re-colonization by peat-forming plants within few years only (Zak et al., 2017). However, top soil removal can be a rather expensive measure and, particularly if the objective of the peatland restoration is to target mitigation of climate change, its carbon footprint needs to be carefully assessed (Zak et al., 2018). Alternatively, harvesting of wetland helophytes and their commercial use (Wichmann 2017) can be another viable option to support the succession toward natural conditions but again longer time periods (>10 years) must be tolerated (Zak et al., 2014).

Acknowledgments

Many thanks to all colleagues of the Department of Chemical Analytics and Biogeochemistry of the Leibniz-Institute of Freshwater Ecology and Inland Fisheries supporting the comprehensive chemical analyses and for their technical assistance during fieldwork. In particular we gratefully acknowledge Jörg Gelbrecht for advice and help with peat and plant sampling. Steffen Vogler, Etienne Brunet, and Angela Krüger are acknowledged for conducting microbial activity tests and phenol analysis. The study was supported by Deutsche Forschungsgemeinschaft (DFG), Grant/Award Number: ZA 742/2-1; Department of Environment of Mecklenburg-Vorpommern, Grant/Award Number: LUNG-230-5325.60-7-34; European Agriculture Guidance and the Guarantee Fund, Grant/Award Number: LUNG-230-5325.60-7-34. Finalization of the study was funded by the European Social Fund (ESF) and the Ministry of Education, Science and Culture of Mecklenburg-Western Pomerania within the scope of the project WETSCAPES (ESF/14-BM-A55-0030/16). VU was financed within the framework of the Research Training Group Baltic TRANSCOAST funded by the DFG under grant number GRK 2000.

References

Appel, H. M. (1993). Phenolics in ecological interactions - the importance of oxidation. J. Chem. Ecol. 19, 1521–1552. doi: 10.1007/BF00984895

Bader, C., Müller, M., Schulin, R., and Leifeld, J. (2018). Peat decomposability in managed organic soils in relation to land use, organic matter composition and temperature. Biogeosciences 15, 703–719. doi: 10.5194/bg-15-703-2018

Bonnett, S. A. F., Maltby, E., and Freeman, C. (2017). Hydrological legacy determines the type of enzyme inhibition in a peatlands chronosequence. Scient. Rep. 7, 1–14. doi: 10.1038/s41598-017-10430-x

Box, J. D. (1983). Investigation of the folin-ciocalteau phenol reagent for the determination of polyphenolic substances in natural-waters. Water Res. 17, 511–525. doi: 10.1016/0043-1354(83)90111-2

Bragazza, L., and Freeman, C. (2007). High nitrogen availability reduces polyphenol content in Sphagnum peat. Sci. Total Environ. 377, 439–443. doi: 10.1016/j.scitotenv.2007.02.016

Bragazza, L., Siffi, C., Iacumin, P., and Gerdol, R. (2007). Mass loss and nutrient release during litter decay in peatland: the role of microbial adaptability to litter chemistry. Soil Biol. Biochem. 39, 257–267. doi: 10.1016/j.soilbio.2006.07.014

Bravo, L. (1998). Polyphenolics, dietary sources, metabolism and nutritional significance. Nutr. Rev. 56, 257–267. doi: 10.1111/j.1753-4887.1998.tb01670.x

Brouns, K., Keuskamp, J. A., Potkamp, G., Verhoeven, J. T. A., and Hefting, M. M. (2016). Peat origin and land use effects on microbial activity, respiration dynamics and exo-enzyme activities in drained peat soils in the Netherlands. Soil Biol. Biochem. 95, 144–155. doi: 10.1016/j.soilbio.2015. 11.018

Brouns, K., Verhoeven, J. T. A., and Hefting, M. M. (2014). Short period of oxygenation releases latch on peat decomposition. Sci. Total Environ. 481, 61–68. doi: 10.1016/j.scitotenv.2014.02.030

Cabezas, A., Gelbrecht, J., Zwirnmann, E., Barth, M., and Zak, D. (2012). Effects of degree of peat decomposition, loading rate and temperature on dissolved nitrogen turnover in rewetted fens. Soil Biol. Biochem. 48, 182–191. doi: 10.1016/j.soilbio.2012.01.027

Couteaux, M. M., Bottner, P., and Berg, B. (1995). Litter decomposition, climate and litter quality. Trends Ecol. Evol. 10, 63–66. doi: 10.1016/S0169-5347(00)88978-8

Cowan, M. M. (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12, 564–582. doi: 10.1128/CMR.12.4.564

Dorrepaal, E., Cornelissen, J. H. C., Aerts, R., Wallen, B., and van Logtestijn, R. S. P. (2005). Are growth forms consistent predictors of leaf litter quality and decomposability across peatlands along a latitudinal gradient? J. Ecol. 93, 817–828. doi: 10.1111/j.1365-2745.2005.01024.x

Erdtman, H. G. H. (1933a). Studies on the formation of complex oxidation and condensation products of phenols. A contribution to the investigation of the origin and nature of humic acid. 1. Studies of the reactivity of simple monocyclic quinones. Proc. Roy. Soc. A 143,177–191. doi: 10.1098/rspa.1933.0212

Erdtman, H. G. H. (1933b). Studies on the formation of complex oxidation and condensation products of phenols. 2. Coupling of simple phenols and quinones to diphenyl derivatives. Proc. Roy. Soc. A 143, 191–222. doi: 10.1098/rspa.1933.0213

Fenner, N., and Freeman, C. (2011). Drought-induced carbon loss in peatlands. Nat. Geosci. 4, 895–900. doi: 10.1038/ngeo1323

Fierer, N., Schimel, J. P., Cates, R. G., and Zou, J. (2001). Influence of balsam poplar tannin fractions on carbon and nitrogen dynamics in Alaskan taiga floodplain soils. Soil Biol. Biochem. 33,1827–1839. doi: 10.1016/S0038-0717(01)00111-0

Franz, D., Koebsch, F., Larmanou, E., Augustin, J., and Sachs, T. (2016). High net CO_2 and CH_4 release at a eutrophic shallow lake on a formerly drained fen. Biogeosciences 13, 3051–3070. doi: 10.5194/bg-13-3051-2016

Freeman, C., Ostle, N., and Kang, H. (2001). An enzymic 'latch' on a global carbon store – A shortage of oxygen locks up carbon in peatlands by restraining a single enzyme. Nature 409:149. doi: 10.1038/35051650

Freeman, C., Ostle, N. J., Fenner, N., and Kang, H. (2004). A regulatory role for phenol oxidase during decomposition in peatlands. Soil Biol. Biochem. 36,1663–1667. doi: 10.1016/j.soilbio.2004.07.012

Fuchs, G., Boll, M., and Heider, J. (2011). Microbial degradation of aromatic compounds – from one strategy to four. Nat. Rev. Microbiol. 9, 803–816. doi: 10.1038/nrmicro2652

Georgé, S., Brat, P., Alter, P., and Amiot, M. J. (2005). Rapid determination of polyphenols and vitamin C in plant-derived products. J. Agric. Food Chem. 53, 1370–1373. doi: 10.1021/jf048396b

Giner-Chavez, B. I., van Soest, P. J., Robertson, J. B., Lascano, C., Reed, J. D., and Pell, A. N. (1997). A method for isolating condensed tannins from crude plant extracts with trivalent ytterbium. J. Sci. Food Agric. 74, 359–368.

Gorham, E. (1991). Northern peatlands role in the carbon cycle and probable responses to climatic warming. Ecol. Appl. 1, 182–195. doi: 10.2307/1941811 Hagerman, A. E. (2002). The Tannin Handbook. Available online at: https://www.users.miamioh.edu/hagermae/ (accessed September 10, 2019).

Hahn-Schöfl, M., Zak, D., Minke, M., Gelbrecht, J., Augustin, J., and Freibauer, A. (2011). Organic sediment formed during inundation of a degraded fen grassland emits large fluxes of CH_4 and CO_2. Biogeosciences 8, 1539–1550. doi: 10.5194/bg-8-1539-2011

Hall, S. J., Treffkorn, J., and Silver, W. L. (2014). Breaking the enzymatic latch: impacts of reducing conditions on hydrolytic enzyme activity in tropical forest soils. Ecology 95, 2964–2973. doi: 10.1890/13-2151.1

Hättenschwiler, S., and Vitousek, P. M. (2000). The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends Ecol. Evol. 15, 238–243. doi: 10.1016/S0169-5347(00)01861-9

Hernes, P. J., and Hedges, J. I. (2000). Determination of condensed tannin monomers in environmental samples by capillary gas chromatography of acid depolymerization extracts. Analyt. Chem. 72, 5115–5124. doi: 10.1021/ac991301y

Hilt, S., Ghobrial, M. G., and Gross, E. M. (2006). In situ allelopathic potential of *Myriophyllum verticillatum* (Halorgagaceae) against selected phytoplankton species. J. Phycol. 42, 1189–1198. doi: 10.1111/j.1529-8817.2006.00286.x

IPCC (2014). 2013 Supplement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Wetlands: IPCC.

Jenkinson, D. S., Adams, D. E., and Wild, A. (1991). Model estimates of CO_2 emissions from soil in response to global warming. Nature 351, 304–306. doi: 10.1038/351304a0

Joosten, H. (2010). The Global Peatland CO_2 Picture: Peatland Status and Drainage Related Emissions in All Countries of the World. Netherlands: Wetland International, Ede.

Joosten, H., Brust, K., Couwenberg, J., Gerner, A., Holsten, B., Permien, T., et al. (2015). MoorFuturesR Integration of Additional Ecosystem Services (Including

Biodiversity) Into Carbon Credits – Standard, Methodology and Transferability to Other Regions. Bonn: Bundesamt für Naturschutz (Federal Ministry for the Environment, BfN).

Koch, M., Koebsch, F., Hahn, J., and Jurasinski, G. (2017). From meadow to shallow lake: monitoring secondary succession in a coastal fen after rewetting by flooding based on aerial imagery and plot data. Mires Peat 19, 1–17. doi: 10.19189/MaP.2015.OMB.188

Kraus, T. E. C., Dahlgren, R. A., and Zasoski, R. J. (2003). Tannins in nutrient dynamics of forest ecosystems - a review. Plant Soil 256, 41–66. doi: 10.1023/A:102620651

Laiho, R. (2006). Decomposition in peatlands: reconciling seemingly contrasting results on the impacts of lowered water levels. Soil Biol. Biochem. 38, 2011–2024. doi: 10.1016/j.soilbio.2006.02.017

Luthria, D. L., and Pastor-Corrales, M. A. (2006). Phenolic acids content of fifteen dry edible bean (*Phaseolus vulgaris* L.) varieties. J. Food Comp. Anal. 19, 205–2011. doi: 10.1016/j.jfca.2005.09.003

Martin, J. S., and Martin, M. M. (1983). Tannin assays in ecological-studies – precipitation of ribulose-1,5-bisphosphate carboxylase oxygenase by tannic acid, quebracho, and oak foliage extracts. J. Chem. Ecol. 9, 285–294. doi: 10.1007/BF00988046

Mole, S., and Waterman, P. G. (1987). A critical analysis of techniques for measuring tannins in ecological-studies II. Tech. Biochem. Def. Tann. Oecol. 72, 148–156. doi: 10.1007/BF00385059

Mueller-Harvey, I. (2001). Analysis of hydrolysable tannins. Anim. Feed Sci. Technol. 91, 3–20. doi: 10.1016/S0377-8401(01)00227-9

Nelson, K. E., Pell, A. N., Doane, P. H., GinerChavez, B. I., and Schofield, P. (1997). Chemical and biological assays to evaluate bacterial inhibition by tannins. J. Chem. Ecol. 23, 1175–1194. doi: 10.1023/B:JOEC.0000

Nyamambi, B., Ndlovu, L. R., Read, J. S., and Reed, J. D. (2000). The effects of sorghum proanthocyanidins on digestive enzyme activity in vitro and in the digestive tract of chicken. J. Sci. Food Agric. 80, 2223–2231. doi: 10.1002/1097-0010(200012)80:15<2223::AID-JSFA768>3.0.CO;2-I

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2017). Vegan: Community Ecology Package, R package version 2.4-5. Available online at: https://CRAN.R-project.org/package=vegan (accessed September 10, 2019).

Pind, A., Freeman, C., and Lock, M. A. (1994). Enzymatic degradation of phenolic materials in peatlands – measurement of phenol oxidase activity. Plant Soil 159, 227–231. doi: 10.1007/BF00009285

Pinsonneault, A. J., Moore, T. R., and Roulet, N. T. (2016). Temperature the dominant control on the enzyme-latch across a range of temperate peatland types. Soil Biol. Biochem. 97, 121–130. doi: 10.1016/j.soilbio.2016. 03.006

Porter, L. J., Hrstich, L. N., and Chan, B. G. (1986). The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25, 223–230. doi: 10.1016/S.0031-9422(00)94533-3

R Core Team (2018). R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing. Available online at: https:// www.R-project.org/ (accessed September 10, 2019).

Rautio, P., Bergvall, U. A., Karonen, M., and Salminen, J. P. (2007). Bitter problems in ecological feeding experiments: commercial tannin preparations and common methods for tannin quantifications. Biochem. System. Ecol. 35, 257–262. doi: 10.1016/j.bse.2006.10.016

Scheffer, B., and Blankenburg, J. (1993). The determination of the bulk-density of peat soils. Agribiol. Res. Zeitschr. Agrarbiol. Agrik. Okol. 46, 46–53.

Schnürer, J., and Rosswall, T. (1982). Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Appl. Environ. Microbiol. 43, 1256–1261.

SPSS Inc. (2005). SPSS Conjoint 14.0 [Software]. Chicago, IL: SPSS, Inc.

Swain, T. (1979). “Tannins and lignins,” in Herbivores: Their Interaction with Secondary Plant Metabolites, eds G. Rosenthal and D. H. Janzen (New York, NY: Academic Press), 657–682.

Van Bodegom, P. M., Broekman, R., Van Dijk, J., Bakker, C., and Aerts, R. (2005). Ferrous iron stimulates phenol oxidase activity and organic matter decomposition in waterlogged Wetlands. Biogeochemistry 76, 69–83. doi: 10.1007/s10533-005-2053-x

Vanbreemen, N. (1995). How Sphagnum bogs down other plants. Trends Ecol. Evol. 10, 270–275. doi: 10.1016/0169-5347(95)90007-1

Verhoeven, J. T., and Liefveld, W. M. (1997). The ecological significance of organochemical compounds in Sphagnum. Acta Bot. Neerl. 46, 117–130. doi: 10.1111/plb.1997.46.2.117

Verhoeven, J. T. A., Arheimer, B., Yin, C. Q., and Hefting, M. M. (2006). Regional and global concerns over wetlands and water quality. Trends Ecol. Evol. 21, 96–103. doi: 10.1016/j.tree.2005.11.015

Von Post, L. (1922). Sveriges geologiska undersöknings torvinventering och några av dess hittills vunna resultat. Sven. Mosskulturfören. Tidskr 1, 1–27.

Wichmann, S. (2017). Commercial viability of paludiculture: a comparison of harvesting reeds for biogas production, direct combustion, and thatching. Ecol. Eng. 103, 497–505. doi: 10.1016/j.ecoleng.2016.03.018

Williams, C. J., Shingara, E. A., and Yavitt, J. B. (2000). Phenol oxidase activity in peatlands in New York state: response to summer drought and peat type. Wetlands 20, 416–421. doi: 10.1672/0277-5212(2000)020[0416:POAIPI]2. 0.CO;2

Williams, C. J., and Yavitt, J. B. (2003). Botanical composition of peat and degree of peat decomposition in three temperate peatlands. Ecoscience 10, 85–95. doi: 10.1080/11956860.2003.11682755

Yavitt, J. B., Williams, C. J., and Wieder, R. K. (2005). Soil chemistry versus environmental controls on production of CH_4 and CO_2 in northern peatlands. Eur. J. Soil Sci. 56, 169–178. doi: 10.1111/j.1365-2389.2004. 00657.x

Yu, Z., Loisel, J., Brosseau, D. P., Beilman, D. W., and Hunt, S. J. (2010). Global peatland dynamics since the last glacial maximum. Geophys. Res. Lett. 37:L13402. doi: 10.1029/2010GL043584

Zak, D., and Gelbrecht, J. (2007). The mobilisation of phosphorus, organic carbon and ammonium in the initial stage of fen rewetting (a case study from NE Germany). Biogeochemistry 85, 141–151. doi: 10.1007/s10533-007-9122-2

Zak, D., Gelbrecht, J., Zerbe, S., Shatwell, T., Barth, M., Cabezas, A., et al. (2014). How helophytes influence the phosphorus cycle in degraded inundated peat soils – implications for fen restoration. Ecol. Eng. 66, 82–90. doi: 10.1016/j.ecoleng.2013.10.003

Zak, D., Goldhammer, T., Cabezas, A., Gelbrecht, J., Gurke, R., Wagner, C., et al. (2018). Top soil removal reduces water pollution from phosphorus and dissolved organic matter and lowers methane emissions from rewetted peatlands. J. Appl. Ecol. 55, 311–320. doi: 10.1111/1365-2664.12931.

Zak, D., Meyer, N., Cabezas, A., Gelbrecht, J., Mauersberger, R., Tiemeyer, B., et al. (2017). Topsoil removal to minimize internal eutrophication in rewetted peatlands and to protect downstream systems against phosphorus pollution: a case study from NE Germany. Ecol. Eng. 103, 488–496. doi: 10.1016/j.ecoleng.2015.12.030.

Zak, D., Reuter, H., Augustin, J., Shatwell, T., Barth, M., Gelbrecht, J., et al. (2015). Changes of the CO_2 and CH_4 production potential of rewetted fens in the perspective of temporal vegetation shifts. Biogeosciences 12, 2455–2468. doi: 10.5194/bg-12-2455-2015

Zak, D., Wagner, C., Payer, B., Augustin, J., and Gelbrecht, J. (2010). Phosphorus mobilization in rewetted fens: the effect of altered peat properties and implications for their restoration. Ecol. Appl. 20, 1336–1349. doi: 10.1890/08-2053.1

Zerbe, S., Steffenhagen, P., Parakenings, K., Timmermann, T., Frick, A., Gelbrecht, J., et al. (2013). Restoration success regarding ecosystem services after 10 years of rewetting peatlands in NE Germany. Environ. Manage. 51, 1194–1209. doi: 10.1007/s00267-013-0048-2

Supplement

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2019.00147/full#supplementary-material

D. Congruent changes in microbial community dynamics and ecosystem methane fluxes following natural drought in two restored fens

Abstract

The frequency and intensity of drought events is expected to increase, with unresolved alterations to peatland methane cycling and the involved microbial communities. Here, we provide the first in situ natural drought study combining microbial and geochemical data, as well as eddy covariance methane flux measurements in two restored, temperate fen peatlands. We used quantitative polymerase chain reaction (qPCR) and high throughput 16S rRNA gene amplicon sequencing of DNA and complementary DNA (cDNA) to determine the abundances and community structure of total and putatively active microbial communities following the 2018 European summer drought. Together with the geochemical and flux data, we compared these results to a non-drought reference dataset. During drought, water level and methane flux rates decreased to a new recent minimum in both fens. This corresponded with pronounced shifts in porewater geochemistry. Microbial community composition in the drought year differed markedly, and was characterized by a greater relative and total abundance of aerobic methanotrophs, and, in one of the two sites, by a decrease in total methanogen abundance. In contrast to the non-drought reference years, type I methanotrophs were clearly more dominant than type II methanotrophs in both fens. cDNA sequencing confirmed the activity of type I methanotrophs during drought, with Methylomonaceae having the highest average relative abundance of bacterial cDNA transcripts. We show that changes in microbial community dynamics, porewater geochemistry, and ecosystem methane fluxes can be substantial following natural drought in restored fens, and provide the first in situ evidence from a natural drought which suggests type I methanotroph populations are more active than type II methanotrophs in response to drought effects. Type I methanotrophs may represent a key microbial control over methane emissions in restored temperate fens subject to natural drought.

Introduction

Peatlands, which store approximately one-third of the world's terrestrial organic carbon (Gorham 1991; Yu et al., 2011), also emit a globally significant amount of the greenhouse gas methane (Bastviken et al., 2011). Peatland ecosystems are particularly vulnerable to drought events, which are likely to increase in frequency and intensity in the upcoming decades (Laiho 2006; IPCC 2014). Methane (CH_4) in peatlands is produced metabolically by methanogenic archaea (methanogens) under water-logged, reducing conditions, when other terminal electron acceptors (TEAs) used in the breakdown of organic matter are depleted (Froelich et al., 1979; Peters and Conrad 1996; Zehnder and Stumm 1988). Methane that does not reach the atmosphere via ebullition, diffusion, or direct transport by plant aerenchyma (Joabsson et al., 1999; Baird et al., 2004) may be oxidized either aerobically or anaerobically by bacteria or archaea (methanotrophs). This mechanism, performed mainly by aerobic methanotrophs in peatlands, can reduce CH_4 emissions substantially (Yavitt et al.,1988). Drought events can decrease peatland CH_4 emissions by reducing water level and altering redox geochemistry (Roden and Wetzel 1996; Knorr and Blodau 2009; Kang et al., 2018). Associated community dynamics of methanogens and methanotrophs, however, are not well understood. *In situ* studies of fen peatlands in this respect are scarce, (Cadillo-Quiroz et al., 2008), although, compared to bogs, fens may be particularly sensitive to drying (Jaatinen et al., 2007; Peltoniemi et al., 2016).

The activity, abundance, and community structure of microbes can be altered by drought effects (Knorr et al., 2008; Kim et al., 2008; Ma et al., 2013; Potter et al., 2017). Oxidative stress can decrease rates of CH_4 production (Jasso-Chávez et al., 2015) and/or cause methanogen mortality (Morozova and Wagner 2007). This is supported by incubation studies and mesocosm experiments that show decreases in CH_4 production (Dowrick et al., 2006; Knorr and Blodau 2009) and methanogen gene abundances (Kim et al., 2008) after short term drought treatments. In two field studies of peatland archaeal communities of the Tibetan plateau, Tian et al. (2012 and 2015) found that drought caused a 10-fold decrease in archaeal abundance (of which the majority were methanogens) and altered community composition. Ma and Lu (2011) also noted a significant decrease in archaeal abundance in a rice paddy soil following short-term drought treatments, citing a decrease in methanogen abundance. In a field manipulation experiment, Peltoniemi et al., (2016) found that both warming and drying decreased methanogen abundances in boreal fens. In some studied locations, however, no changes in methanogen abundance and community structure were found (e.g. Kim et al., 2008; Peltoniemi et al., 2016), suggesting drought-induced changes to microbial community dynamics may differ among individual peatlands.

Whether aerobic methanotrophic communities can maintain function under increasing frequency and intensity of natural drought is still not clear (Ho et al., 2016a). Drought effects on aerobic methanotrophic communities in peatlands have been studied mostly in controlled incubation experiments (e.g. Henckel et al., 2001; Ma and Lu 2011; Collet et al., 2015; Ho et al., 2016a; van Kruistum et al., 2018). Some aerobic methanotrophs benefit from water level reduction and modest soil drying (Henckel et al., 2001; Ma and Lu 2011; Peltoniemi et al., 2016). Aerobic proteobacterial methanotrophs are divided into types I (gammaproteobacteria) and II (alphaproteobacteria) based on phylogenetic and biochemical distinctions (Knief 2015), and an increasing number of studies suggest a differential response to drying events among aerobic methanotroph types (Collet et al., 2015; Ho et al., 2016a; Peltoniemi et al., 2016).

Incubation studies suggest type I methanotrophs can adjust more readily to moisture and temperature fluctuations, and are able to rapidly increase activity and population size once suitable conditions return (Henkel et al., 2001; Bodelier et al., 2012; Ho et al., 2013a; Ho et al., 2013b; Pan et al., 2014; Collet et al., 2015; Ho et al., 2016a; Ho et al., 2016b). Though ubiquitous in peatlands, type II methanotroph populations are thought to be comparatively stable under changing temperature and moisture regimes (Henckel et al., 2001; Bodelier et al., 2011; Collet et al., 2015) as a consequence of their different life strategy as stress-tolerators, compared to type I methanotrophs, which have been described broadly as competitor-ruderals (Ho et al., 2013a). This has not yet been investigated in fens under natural drought. However, in their field manipulation experiment, Peltoniemi et al. (2016) found a combination of warming and drying decreased the abundance of some type I methanotrophs, while differences in the abundance of type II methanotrophs could only be explained by depth, and not by warming and/or drying treatments.

The 2018 European drought (Hanel et al., 2018) provided the opportunity to examine microbial community and CH_4 emission dynamics in a natural setting. According to the European Centre for Medium-Range Weather Forecasts, the near-surface air temperature anomaly from April to August 2018 in Europe was greater than in any year since 1979, with the Baltic Sea region experiencing the highest anomalies (Magnusson et al., 2018). Data from the National Oceanic and Atmospheric Administration place 2018 as the warmest summer in Europe on record since 1910 (Global Climate Report 2020). From April to September 2018, monthly precipitation averaged across Germany ranged from 25.6 % (April) to 52.7 % (July) less than the 1981-2010 reference period average (Deutscher Wetterdienst 2020). Such drastic deviations are likely to be reflected in fen CH_4 cycling and the involved microbial communities, but most existing studies are experimental (e.g. Kim et al., 2008; Tian et al., 2015; Peltoniemi et al., 2016) or do not examine methanogens and methanotrophs specifically.

Total microbial, methanogen, and methanotroph community dynamics have not yet been studied together with CH_4 emissions and porewater geochemistry in restored fens affected by natural drought. Therefore, during the 2018 European summer drought, we collected peat and porewater samples from two previously studied fens in Northeastern Germany. We analyzed peat microbial community structure and relative abundances using 16S rRNA gene sequencing, and determined total microbial, methanogen, and bacterial methanotroph abundances using quantitative PCR (qPCR). We include porewater geochemical analyses, as well as ongoing CH_4 flux and water level measurements, and compared this dataset to a similar dataset from a non-drought year published by Wen et al. (2018). In the drought year only, we employed reverse transcription of total RNA and subsequent sequencing of complementary DNA (cDNA) to develop a community profile of active microbes. We aimed to elucidate methanogen and methanotroph community dynamics associated with decreasing CH_4 emissions in restored fens affected by natural drought. Along with a decrease in CH_4 flux rates, we hypothesized a decrease in the abundance of methanogens, as well as shifts in microbial community composition, with a greater representation of aerobic taxa during drought, specifically methanotrophs. Furthermore, we expected the community profile of microbes active during drought to be dominated by aerobic taxa.

Materials and Methods

Study sites

For a detailed description of the study sites, see Wen et al. (2018). Briefly, the Hütelmoor is a minerotrophic fen of approx. 360 ha located at the Baltic Sea in NE Germany (Fig. D-1a and b). In the past, then fen received brackish water inflows from occasional storm surges, though the last brackish water intrusion event happened more than 20 years ago, in 1995. Since then, the brackish sulfate pool of the upper soil horizons has been fully depleted, so that contemporary conditions for methane cycling correspond to those of freshwater peatlands (Koebsch et al., 2019; Wen et al., 2018). The second site, Zarnekow, is a riparian fen of approx. 500 ha, located in the valley of the river Peene in NE Germany (Fig. D-1a and c). Both fens were drained for agricultural purposes and were restored in 2005 (Zarnekow) and 2010 (Hütelmoor) by permanently raising water levels above peat surface. After rewetting, average CH_4 fluxes increased dramatically (Augustin and Chojnicki 2008; Hahn et al., 2015; Franz et al., 2016; Wen et al., 2018).

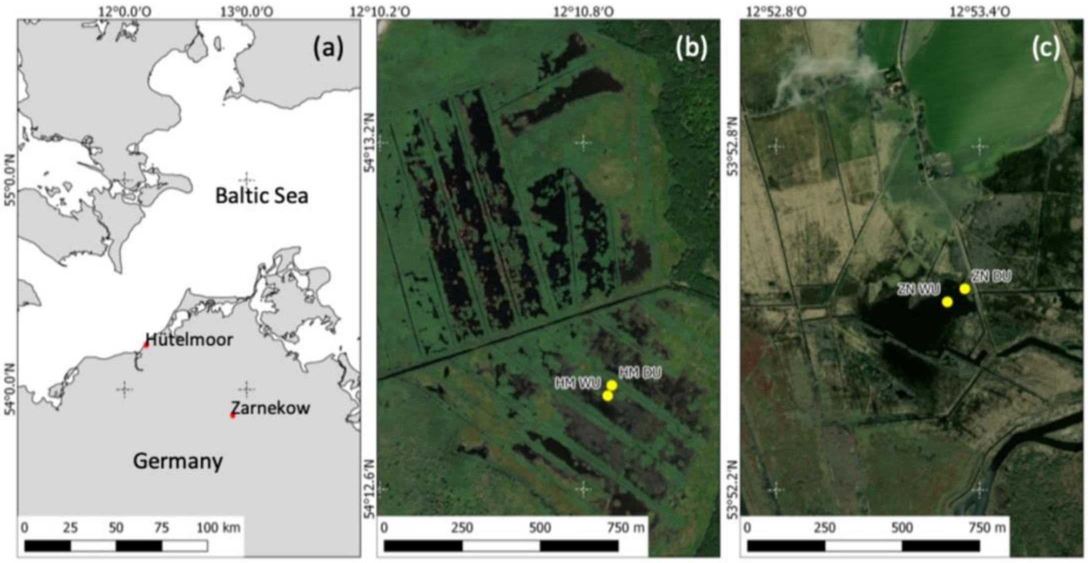


Figure D-1 – Location of the study sites in Northeastern Germany (a) and sampling locations in the coastal fen, the Hütelmoor (b), and the riparian fen, Zarnekow (c). Two sampling points were located in each of the wet unvegetated (WU) and dry unvegetated (DU) subsites. Aerial images were taken in May 2018, before average water levels decreased in the fens.

Peat and porewater collection (drought year)

Peat and porewater samples for the drought year were collected on August 30 and September 12, 2018 in the Hütelmoor and Zarnekow, respectively. At this time, the average water level in both fens was visibly lower, nevertheless, the distinct microtopography led to a patchy mosaic of dry spots, and small ponds with water depths ~10 cm or less. Samples were collected in both the dry and wet subsites to assess whether spatial patterns in hydrology were reflected in microbial community dynamics and geochemistry. Sampling was conducted in non-vegetated sites to exclude the potential influence of plants and rhizosphere effects on CH_4 cycling and involved microorganisms. In each subsite, duplicate peat cores were collected for analyses of microbial community composition and concentrations of dissolved CH_4 and carbon dioxide (CO_2). Surface samples were collected for microbial analyses only. A third and fourth core (one from each subsite) were collected for bulk density and porewater geochemical analyses, respectively. All peat cores were semi-cylindrical (5 cm width x 50 cm depth) and were collected with a Russian D-corer (Vleeschouwer et al., 2010). The cores were split into 10 cm depth sections and the depth sections 0–10, 20–30, and 40–50 cm were analyzed for this study.

Peat samples for microbial analyses were collected using sterile equipment, placed in 15 ml Falcon Tubes and immediately placed in a dry-shipper that was pre-cooled with liquid nitrogen. Samples were stored in the lab at -80 °C for approximately one week until nucleic acid extraction. For dissolved CH_4 and CO_2 concentrations and their $^{13}C/^{12}C$ isotopic composition, a 3 ml peat "plug" was collected with a tip-cut 3 ml syringe from each depth section and placed in a 20 ml vial containing 5M NaCl solution. The vials were closed immediately with butyl rubber stoppers and aluminum crimp caps with no headspace, and then stored upside down until processing. After collecting a peat plug of a known volume for bulk density analysis (again using a tip-cut syringe), peat samples for porewater geochemical analyses were immediately packed in air-tight aluminum bags and sealed with plastic clips. Upon returning to the lab several hours later, the aluminum bags were flushed with nitrogen gas, completely degassed using a vacuum pump, heat-sealed, and stored at 4°C until analysis.

Sample and data collection (non-drought year)

A detailed description of the study design and methods from the non-drought year is provided in Wen et al., (2018), but a summary of this, and how the data were applied in this study, are provided here. Peat and pore water were collected in four locations in the Hütelmoor and five locations in Zarnekow in October 2014 and July 2015, respectively. At the time of sampling, the sampling locations were fully inundated since rewetting. From this timepoint, two sampling locations from each fen were selected to compare to the data collected in the drought year. This was done to ensure that data from similar locations in the fens were compared. In both the drought and non-drought years, peat collection methods for microbial analyses were conducted with the same equipment and analyzed with the same protocols. Porewater collection differed between the drought and non-drought years. In the non-drought year, porewater was collected from permanently installed porewater dialysis samplers using a syringe. In the drought year, peat samples were collected, from which porewater was extracted using a hydraulic pore water press. Dissolved CH_4 and dissolved CO_2 concentrations were determined using the same equipment, while concentrations of TEAs and isotopic analyses were conducted on different equipment. Sequencing data from the non-drought year were initially analyzed and published using OTU clustering methods (Wen et al., 2018). For the present study, amplicon sequence variant methods were employed, and sequencing data from the non-drought year were therefore reanalyzed accordingly. RNA extraction, reverse transcription, and subsequent cDNA sequencing were conducted in the drought year only.

Water level and methane flux measurements

Water level in Zarnekow was derived with a sonic ranging sensor SR50A (Campbell Scientific, Canada) that measured the distance from a fixed platform down to the water surface. The distance measured by the SR50A was corrected for temperature effects and converted to water levels relative to the sediment surface. Water level in the Hütelmoor was derived from pressure measurements using a HOBO 13-Foot Depth Titanium Water Level Data Logger (Onset, Bourne, USA) after barometric pressure correction, and was referenced to the mean surface level. In this study, inundated conditions with water levels below the peat surface are denoted with a negative sign.

CH_4 fluxes were measured with the eddy covariance approach, which provides a continuous time series of half-hourly gas fluxes on ecosystem scale. In the Hütelmoor, the measurement setup comprised of two open-path infrared gas analyzers (LI-7500 and LI-7700, both LI-COR, Lincoln, NE, USA), and a three-dimensional sonic anemometer (CSAT3, Campbell Scientific, Logan, UT, USA) for wind velocities and sonic temperature. All signals were recorded by a CR3000 Micrologger (Campbell Scientific, Logan, UT) with a scan rate of 10 Hz. In Zarnekow, the setup for CH_4 flux measurements comprised of an open-path CH_4 analyzer (LI-7700, LI-COR, Lincoln, NE, USA) and a closed-path Fast Greenhouse Gas Analyzer (FGGA EP, Los Gatos Research). The sonic anemometer is a Gill HS-50 (Gill, Lymington, Hampshire, UK) and raw data were recorded with a LI-7550 digital data logger system (LI-COR Biogeosciences, Lincoln, NE, USA) at 20 Hz in half-hourly files. Though instrumentation and configuration differed slightly between both sites, each of the measurement setups is well in line with the default practice used within the eddy covariance community. Half-hourly net CH_4 fluxes were processed with the software EddyPro version 6.0.0 (LI-COR, Lincoln, NE, USA). Data gaps were imputed with the same artificial neural network approach for both sites.

Bulk density and porewater chemistry

Water content was determined by dividing the oven dry weight of a given peat sample over the wet weight. Bulk density (g dry weight cm^{-3}) of each depth section was calculated by dividing the oven dry weight of a peat sample by the initial volume. Porewater for geochemical (pH and anion) analyses was extracted from the peat samples using an IODP-style titanium pore water squeezer (Manheim 1966) in a 22-ton hydraulic press (Carver, Wabash, USA). The extractors were fitted with 0.22 µm pore size filters. Anions nitrite (NO_2^-), nitrate (NO_3^-), and sulfate (SO_4^{2-}) were quantified on an ion chromatograph system with an S5200 sample injector, a 3.0 mm×150 mm SykroGel A 01 column, a S3115 conductivity detector (all SYKAM Chromatographie Vertriebs GmbH, Germany), and a SeQuant SAMS anion IC suppressor (Merck KGaA, Germany). The eluent (6 mM Na_2CO_3 and 90 µM NaSCN) had a flow rate of 1 mL min^{-1}. Column oven temperature was 50 °C, and the injection volume was 50 µl.

Dissolved methane and carbon dioxide

In the lab, a 3 mL gas headspace was created in each porewater sample vial by addition of ultrapure helium using two sterile syringes. After being allowed to equilibrate for two weeks (upside down to avoid gas leakage) samples were analyzed on a 7890A gas chromatograph (GC) system (Agilent Technologies, Germany) equipped with a flame ionization detector and a Carboxen PLOT Capillary Column or HP-Plot Q (Porapak-Q) column. Injection volume was 250 µL. Dissolved CH_4 and CO_2 concentrations were calculated and converted to micromolar values from the equation $\frac{G*H}{T*R*V*P} * 1000$ where G = headspace gas concentration (ppm), H = headspace volume (3 mL), T = absolute temperature (301.15 °K), R = universal gas constant (0.082 $L \cdot atm \cdot K^{-1} \cdot mol^{-1}$), V = peat volume (3 mL), and P = peat porosity. Porosity was calculated from the measured bulk density.

Isotopic composition of methane and carbon dioxide

The $^{13}C/^{12}C$ isotopic composition of CH_4 and CO_2 was determined using cavity ring-down spectroscopy (CRDS; PICARRO G2201-i PICARRO Instruments, Sunnyvale, CA, United States) coupled to a Small Sample Isotope Module (SSIM) in order to measure small gas sample volumes (~20 ml). To avoid interference with hydrogen sulfide present in samples, 1 ml of a saturated Zn-acetate solution was added to precipitate hydrogen sulfide as ZnS. As the measurement range of the instrument is 300-2000 ppm for CO_2 and 2.5-2000 ppm CH_4, small gas samples from headspace vials were taken (100 µL) and analyzed on a GC equipped with FID and TCD (GC 2010, Shimadzu, Kyoto, Japan) to determine a suitable dilution in synthetic air for isotope measurements to fall in the acceptable range. The calibration for CH_4 was performed using a working standard of 1000 ppm (-42.48 ‰) and four standards of 2500 ppm (-38.30, -54.45, -66.50 and -69.00 ‰). CO_2 calibration was performed using a standard of 1000 ppm with known isotopic signature of -31.07 ‰ and dilution of pure CO_2 with signatures of -27.10 and -4.55 ‰, respectively. All gas standards had been calibrated against reference materials from IAEA (RM8562) using elemental analysis coupled to isotope ratio mass spectrometry (EA 3000, Eurovector, Redavalle, Italy; Horizon, NU Instruments, Wrexham, UK) or were provided from Air Gas (Air Liquide, Plumsteadville, PA, USA) or from Isometric Instruments (GASCo, Victoria, BC, Canada) with certificates. Isotope values are expressed in units of per mill (‰) in the typical δ-notation vs. V-PDB.

DNA extraction and sequencing

Genomic DNA was extracted from 150 to 200 mg of peat soil per sample using an EurX GeneMATRIX soil DNA Purification Kit. DNA concentrations were quantified using a Nanophotometer P360 (Implen GmbH, Munich, Germany). Polymerase chain-reaction (PCR) analysis of bacterial and archaeal 16S rRNA genes was performed as detailed in Wen et al., (2018). Primer combinations for archaea were S-D-Arch-0349-a-S-17/S-D-Arch-0786-a-A-20 (Takai and Horikoshi 2000) and S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 (Herlemann et al., 2011) for bacteria. Illumina HiSeq sequencing was performed by Eurofins Genomics using 300 bp paired-end mode and a 20 % PhiX Control v3 library to offset the effects of low-diversity sequence libraries.

RNA extraction and cDNA synthesis

Total RNA was extracted using the Qiagen RNeasy PowerSoil Total RNA kit. The resulting RNA was purified using a TURBO DNA-free kit (Invitrogen). Complete digestion of DNA was verified using RNA as non-template control in conventional 16S rRNA PCR reactions as described in Liebner and Svenning (2013). Quality of the RNA was checked with an Agilent 2100 Bioanalyzer (Agilent Technologies, US). Reverse Transcriptase polymerase chain reaction was conducted on total RNA according to in house protocol. First, 10 µl sterile distilled water, 1µl 10mM dNTP mix (Invitrogen), 1µl pd(N)$_6$ Random Hexamer (GE Healthcare), and 1µl of sample were combined in a PCR tube on ice (final volume 13 µl). The mixture was heated at 65°C for 5 minutes in a PCR machine (Bio-Rad) and immediately chilled on ice. The contents of the tubes were spun down and the following reagents were then added: 1 µl sterile distilled water, 1 µl M DTT, 1 µl Superscript III Reverse Transcriptase (Invitrogen), and 4 µl 5 x First Strand Buffer. The reagents were mixed gently by pipetting up and down, and were incubated first at 25°C for 5 minutes (because random primers were used), then 50 °C for 60 minutes, and finally deactivated by heating at 70 °C for 15 minutes. The resulting cDNA was then used as a template for PCR amplification. PCR amplification was performed on bacterial and archaeal cDNA using the same primer combinations as mentioned above.

Taxonomic analyses

Cutadapt (Martin 2011) was used to demultiplex the NGS libraries. Primer sequences were detected while allowing for a maximum error rate of 10 % whereas sample barcodes were not allowed to show any sequencing error while having a high-quality score (Q25). The DADA2 pipeline (Callahan et al., 2016) was applied to process the sample sequence libraries by truncating

(250 bp forward reads; 200 bp reverse reads) and filtering read sequences. A library-specific error model was generated and used for dereplication, for sample inference and for the merging of read pairs. Resulting sequences were required to have a minimum length of 200 bp and their orientation was standardized by calculating the hamming distance of the sequences and their reverse complements. De novo chimera removal was applied to the resulting sequence table. The resulting amplicon sequence variants (ASVs) were assigned to the SILVA taxonomy (v132) (Quast et al., 2013) by applying vsearch (Rognes et al., 2016) as implemented in the QIIME2 pipeline (Bolyen et al 2018). In order to account for different sequencing depths, the relative abundances of the ASVs were used for the visualization and comparison of the microbial communities.

Quantification of 16S rRNA, mcrA, and pmoA gene copy numbers

Quantitative polymerase chain reaction (qPCR) was performed on a Bio-Rad CFX instrument (Bio-Rad, Munich, Germany) using the SYBR green method for determination of 16S *rRNA* (total bacterial), *mcrA* (methanogenic), and *pmoA* (bacterial methanotrophic) gene copy numbers. A detailed description of the procedure can be found in Wen et al., (2018). *Methylocella* spp. were not present in the sequencing data and the marker gene *mmoX was* therefore excluded from qPCR analysis.

Statistical methods and data visualization

All statistics and data visualization were done using R (R Core Team 2019). The Mann-Whitney test was employed to examine differences in geochemical parameters and microbe abundances in drought versus non-drought years, as well as subsites. NMDS ordinations were constructed using the function metaMDS of R package vegan (Oksanen et al., 2019) to visualize dissimilarity in microbial community composition at the family level among subsites and before and after drought. Bubble plots were constructed at the family level to examine differences in relative abundances of methanogens and methanotrophs between drought and non-drought conditions, as well as to visualize the active communities of both groups.

Accession numbers

The 16S rRNA gene sequence data from the non-drought year were deposited at EBI under the BioProject PRJNA356778. The Hütelmoor sequence read archive accession numbers are SRR5118134-SRR5118155 and SRR5119428-SRR5119449 for bacterial and archaeal sequences, respectively. The Zarnekow accession numbers are SRR6854018-SRR6854033 and SRR6854205-SRR6854220 for bacterial and archaeal sequences, respectively. Raw sequencing data from the drought year are available at the European Nucleotide Archive under BioProject accession number PRJEB38162 and sample accession number ERS4542720-ERS4542857.

Results

Methane emissions and water level

Methane emissions and water level dynamics were similar among the fens in the drought year (Fig. D-2). Water levels were high in April 2018, at 0.6 and 0.8 m above the surface in the Hütelmoor and Zarnekow, respectively (Fig. D-2c and d). However, water levels dropped steadily from May on and reached surface level between end of July/beginning of August. At this time, CH_4 emissions in both fens peaked at 0.90 (Hütelmoor) and 0.87 (Zarnekow) g m^{-2} d^{-1}, then decreased sharply afterwards (Fig. D-2a and b). For the rest of the year, CH_4 emissions remained low with median fluxes of 0.02 and 0.04 g m^{-2} d^{-1}. Although water levels rose above ground surface in November, CH_4 emissions were lower than the same time period of the three previous years. This held particularly for the Hütelmoor, where median November CH_4 fluxes reached only 3 % of the magnitude of the previous 3 reference years.

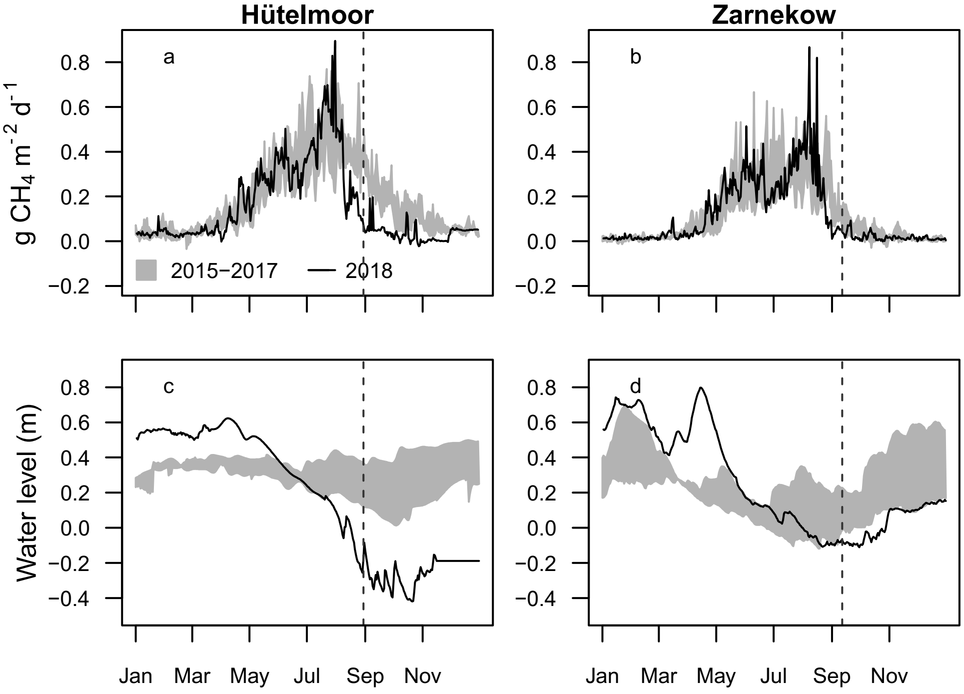


Figure D-2 – Annual trends in eddy covariance-determined methane flux rates (a and b) and water level (c and d) for the Hütelmoor and Zarnekow. Grey areas represent the range of measured values for the three years prior to the drought year (2015–2017), while the solid black line represents the drought-year average (2018). Dotted lines indicate the time of sampling in the drought year.

Porewater geochemistry

Dissolved SO_4^{2-} concentrations were higher during drought in both fens ($p = 0.02$, Table 1), while NO_3^- concentrations did not differ among the drought and non-drought years ($p > 0.05$). In contrast to the non-drought year, where NO_3^- was only detected in a few surface samples, NO_3^- was detected in nearly every sample during drought. Dissolved CH_4 concentrations were significantly lower in both fens during the drought (Table D-1). Dissolved CO_2/CH_4 ratios were higher in the dry subsites than wet subsites, ranging from 31 to 1,912 and 1 to 18 in dry and wet subsites respectively (Table D-1). $\delta^{13}C$-CH_4 values ranged from 67.5‰ to -52.5‰ Slightly higher $\delta^{13}C$-CH_4 values were measured in the dry subsites of Hütelmoor (-52.5‰) and Zarnekow (-56.7‰) at the 20–30 and 40–50 cm depths, respectively. Except for the 0–10 cm depth in the Hütelmoor and 40–50 cm depth in Zarnekow, where $\delta^{13}C$-CO_2 values were -13.3‰ and -14.8‰, respectively, $\delta^{13}C$-CO_2 values in the dry subsites were around -20‰. In the wet subsites, $\delta^{13}C$-CO_2 values were higher (Table D-1). Gas concentrations were too low to determine isotopic values for depths of 0 to 40 cm in the dry subsites in Zarnekow, and isotopic values for the 40–50 cm depth section in Zarnekow could only be obtained for one core (Table D-1).

Table D-1 – Total microbial, methanogen, and methanotroph abundances as well as geochemical parameters for dry and wet subsites during the 2018 summer drought. Values shown are averages calculated from duplicate core

	Depth	16S rRNA	*mcrA*	*pmoA*	Dissolved CH_4	Dissolved CO_2	CO_2/CH_4	$\delta^{13}C-CH_4$	$\delta^{13}C-CO_2$	NO_3^-	NO_2^-	SO_4^{2-}
	cm	copies g dry peat^{-1}	copies g dry peat^{-1}	copies g dry peat^{-1}	µM	µM		V-PDB	V-PDB	mM	mM	mM
Hütelmoor Dry	Surface	3.6 x 10^{10}	6.5 x 10^{8}	4.8 x 10^{7}	-	-	-	-	-	-	-	-
	0-10	2.0 x 10^{10}	7.2 x 10^{8}	2.7 x 10^{7}	8	1194	121	-60.9	-21.0	6.20	-	28.5
	20-30	1.2 x 10^{10}	2.0 x 10^{8}	1.1 x 10^{7}	12	1147	94	-52.5	-21.6	-	-	19.0
	40-50	1.3 x 10^{10}	1.0 x 10^{8}	9.2 x 10^{6}	6	739	69	-63.3	-19.6	3.51	2.75	35.7
Hütelmoor Wet	Surface	2.2 x 10^{10}	1.5 x 10^{9}	1.2 x 10^{8}	-	-	-	-	-	-	-	-
	0-10	3.3 x 10^{10}	8.7 x 10^{8}	7.8 x 10^{7}	248	1818	5	-64.6	-13.3	0.03	0.09	0.60
	20-30	2.6 x 10^{10}	3.0 x 10^{6}	2.7 x 10^{7}	80	777	17	-66.3	-19.8	0.12	0.15	0.44
	40-50	8.9 x 10^{9}	4.2 x 10^{6}	7.5 x 10^{6}	35	1231	18	-67.5	-20.9	3.57	0.22	38.6
Zarnekow Dry	Surface	2.0 x 10^{10}	1.6 x 10^{8}	3.6 x 10^{7}	-	-	-	-	-	-	-	-
	0-10	2.2 x 10^{10}	2.4 x 10^{8}	1.5 x 10^{7}	0.4	1072	1912	-	-	0.09	0.12	1.49
	20-30	1.8 x 10^{10}	1.2 x 10^{8}	2.3 x 10^{7}	4	1080	276	-	-	0.19	0.20	1.16
	40-50	7.3 x 10^{9}	4.5 x 10^{7}	8.5 x 10^{6}	27	666	31	-56.7	-14.8	0.10	0.07	0.33
Zarnekow Wet	Surface	1.8 x 10^{9}	1.2 x 10^{8}	1.0 x 10^{7}	-	-		-	-	-	-	-
	0-10	3.7 x 10^{11}	6.1 x 10^{7}	8.5 x 10^{6}	538	332.4	1	-62.5	-10.2	-	-	0.65
	20-30	3.2 x 10^{10}	4.4 x 10^{7}	7.1 x 10^{6}	382	651.0	1	-65.0	-10.8	0.53	0.38	33.90
	40-50	5.5 x 10^{10}	4.9 x 10^{6}	2.9 x 10^{6}	226	667.4	6	-62.9	-13.2	-	-	2.25

16S rRNA, mcrA, and pmoA gene copy numbers

During drought, total microbial abundance based on 16S *rRNA* gene copy numbers ranged from 1.8×10^9 to 3.7×10^{11} and was—compared to the non-drought year—slightly higher in Zarnekow ($p = 0.03$), but did not differ in the Hütelmoor (Table 1). Total methanogen abundance based on *mcrA* gene copy numbers ranged from 3.0×10^6 to 1.5×10^9 copies g dry peat^{-1} and was significantly lower in Zarnekow during drought ($p = 9.8 \times 10^{-5}$), but similar among the drought and non-drought years in the Hütelmoor ($p = 0.4$). Total bacterial methanotroph abundance based on *pmoA* gene copy numbers, which ranged from 2.9×10^6 to 1.2×10^8 copies g dry peat^{-1}, was significantly higher in the Hütelmoor during the drought ($p = 2.5 \times 10^{-2}$) but did not differ in Zarnekow ($p = 0.15$). Bacterial methanotroph abundances were significantly higher in the dry subsites in Zarnekow ($p = 0.007$), but no other significant differences in gene abundances were found among dry and wet subsites.

Overall community composition of bacteria and archaea

According to the NMDS ordination, bacterial community composition based on 16S *rRNA* gene sequencing differed among the fens in both the drought and non-drought years (Fig. D-3). In the drought year, bacterial community composition differed from the non-drought year, but the patterns of differentiation were not consistent among the fens, with community composition diverging in Zarnekow and converging in the Hütelmoor. Surface samples showed the strongest differentiation in community composition among drought and non-drought years. Archaea community composition also differed among the fens at both time points (Fig. D-3). Community composition converged strongly in the Hütelmoor following drought, but there was little overall difference among the dry and wet subsites and time points. In Zarnekow, archaeal community composition during drought clearly differed from the non-drought year.

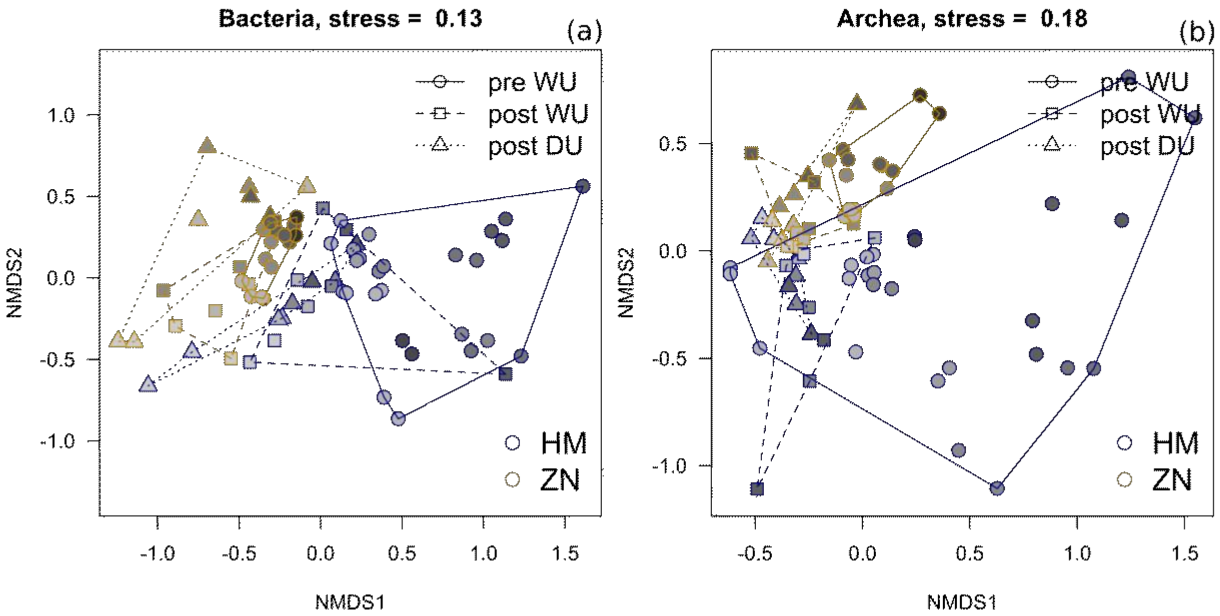


Figure D-3 – NMDS ordinations showing bacterial (a) and archaeal (b) community composition in the non-drought year (pre WU) and the wet and dry subsites of the drought-year (post WU and post DU, respectively). Points represent distinct microbial communities, with the darkest points representing the microbial community composition at depth (40–50 cm), and the lightest points representing surface sample communities. Polygons indicate clusters within the respective time points. The dotted and dashed polygons refer to samples from the drought-year, while the solid polygons refer to the non-drought year. Blue highlights samples from the Hütelmoor (HM) and yellow highlights samples from Zarnekow (ZN).

Methanotroph community composition and relative abundances based on 16S rRNA gene sequencing and cDNA sequencing

In contrast to the non-drought year, the type I aerobic methanotrophs of the order Methylococcales clearly dominated the methanotrophic community during drought. Type I methanotrophs of Methylococcales were detected in all but four samples, and represented an average of 1.1 % of total bacterial community, with a maximum of 3 % in dry subsites, and 6 % in wet subsites. In particular, there was a pronounced increase in the distribution and relative abundance of family Methylomonaceae, but Methylococcaceae were also detected in a greater number of samples in the drought year (Fig. D-4 a and b). The distribution and abundance of type II methanotrophs within Beijerinckiaceae was similar among the drought and non-drought years (Fig. D-4 a and b). *Methylomonas* was the most abundant genus, followed by *Crenothrix*, *Methylobacter, Methylococcus*, "*Candidatus* Methylospira", and *Methyloparacoccus*. Type II methanotrophs were represented mainly by *Methylocystis*, and were only a small portion of the Beijerinckiaceae taxa detected, making up only 0.1 % of bacterial community or less. Methylomirabilaceae, the family containing proposed anaerobic methanotroph (ANME) "*Candidatus* Methylomirabilis" (*Ca*. Methylomirabilis), was detected in many samples from both time points

(Fig. D-4 a and b), and was represented almost exclusively by the uncultured group Sh765B-TzT-35. *Ca*. Methylomirabilis was detected in deeper peat (20–50 cm depth) at both time points in Zarnekow where it represented 0.01 to 0.05 % of bacterial community, but was low in abundance (≤ 0.05 %) and not detected in the Hütelmoor (data not shown).

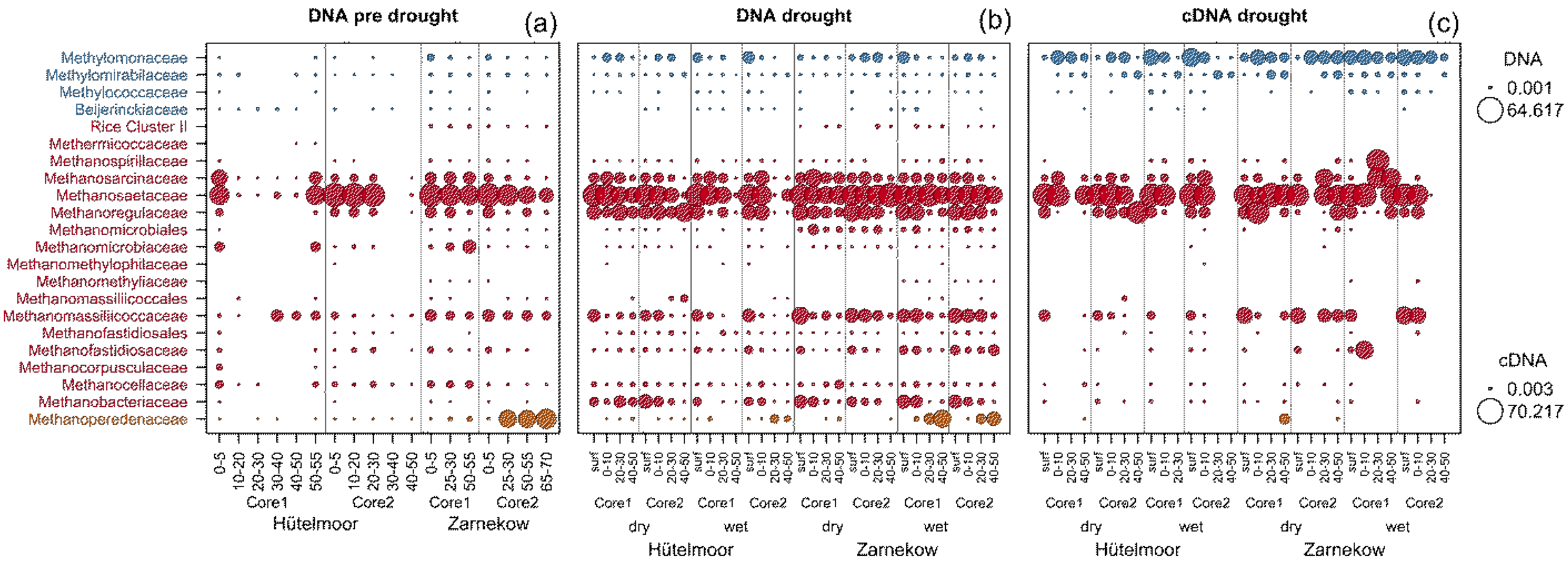


Figure D-4 – Bubble plots showing the relative abundances (in %) of aerobic methanotrophs (blue), methanogens (red), and ANME (orange) for the non-drought (a) and drought years (b and c). Plots a and b were constructed from sequenced DNA in the non-drought and drought year, respectively, while plot c was constructed from sequenced cDNA from the drought-year only. The abundances shown for aerobic methanotrophs are calculated relative to total bacterial community, while the abundances of methanogens and ANME are calculated relative to total archaeal community.

Along with other aerobic bacteria (e.g. Arenicellaceae and Nitrosomondaceae), type I methanotrophs (mostly Methylomonaceae) were active during drought, and dominated the active community profile (Supplemental data, Fig. D-S2). Within the bacterial community, type I methanotrophs of Methylomonace*a*e had the highest average (8 %) and maximum (26 %) relative abundance of all methanotrophs (Fig. D-4 c). Type II methanotrophs (*Methylocystis*) were active in just five samples from both fens, with a maximum relative abundance of 0.09 % of bacteria. *Ca*. Methylomirabilis was detected in just two deeper samples in Zarnekow. However, Methylomirabilaceae*,* and in particular group Sh765B-TzT-35 (data not shown), represented a significant portion of the cDNA-determined community (Fig. D-4 c).

Methanogen community composition and relative abundances based on DNA and cDNA sequencing

In the drought year, methanogens remained the most abundant archaeal group, along with Crenarchaeota (Supplemental data, Fig. D-S3). During drought, Methanosaetaceae accounted for 23–97 % of archaeal community in the DNA-based, which was similar to relative abundances measured in the non-drought year (Fig. D-4 a and b). The acetoclastic methanogens Methanosaetaceae (also known as Methanothrix) dominated the archaeal community, along with the metabolically diverse Methanosarcinaceae, the H_2/formate-utilizing Methanoregulaceae, and the hydrogenotrophic Methanomassiliicoccaceae (Supplemental data, Fig. D-S3). This was also reflected in the active community profiles (Fig. D-4 c; Supplemental data, Fig. D-S4). Taxa that were detected in the DNA-based community profiles were not always detected in the active community profiles at similar depths (this was true for both bacteria and archaea), suggesting the presence of inactive microorganisms (Fig. D-4 a–c, Supplemental data, Figs. S1-4). In the DNA-based community profile, the ANME family Methanoperedencaceae was detected in nearly all depths in both fens, but was relatively most abundant in deeper peat (Fig. D-4 a and b). This family was represented entirely by the anaerobic methanotroph "*Candidatus* Methanoperedens nitroreducens" (*Ca*. M. nitroreducens). At depths of 20 to 50 cm, "*Ca*. M. nitroreducens" represented up to 2 % of archaea in the Hütelmoor and up to 18 % in Zarnekow. In the active community profile, "*Ca*. M. nitroreducens" was detected in one sample from Hütelmoor at 40–50 cm, where it represented 0.2 % of archaeal cDNA sequences. In Zarnekow, it was detected at 40–50 cm in both cores from the dry subsites with relative abundances of 0.4 and 4 % (Fig. D-4). This was additionally the only location and depth where "*Ca*. Methylomirabilis" (group Sh765B-TzT-35) cDNA sequences were detected. The organism is understood to use NO_2^- produced by "*Ca*. M. nitroreducens" via anaerobic CH_4 oxidation coupled to NO_3^- reduction.

Discussion

Drought methane emissions and site conditions

In both fens, a substantial peak in CH_4 emissions occurred in August of the drought year, as water level approached the peat surface. The magnitude of the peak exceeded CH_4 emission rates of the previous 3 years. Previous studies have reported a brief pulse in CH_4 emissions associated with water level reduction in peatlands, likely because of degassing due to reduced hydrostatic pressure (Moore et al., 1990; Dinsmoore et al., 2009) and/or increasing peat temperatures associated with the heat wave, as warming can enhance CH_4 production significantly as long as fen peats are moist (Turetsky et al., 2008). Once water levels reached below the peat surface, CH_4 emissions were severely diminished (below 0.2 g CH_4 m^{-2} d^{-1}). Emissions remained low into late autumn despite increasing water levels, signifying a biogeochemical legacy of draining. This observation could be the result of drought-induced replenishment of alternative TEAs (Estop-Aragonés et al., 2013; Clark et al., 2020). During drought, we measured higher SO_4^{2-} concentrations and detected NO_3^- at greater depths and in a larger number of samples compared to the non-drought year. With these (and other) alternative electron acceptors, various other microorganisms may outcompete methanogens for substrates (Kristjansson and Schönheit 1983; Scholten et al., 2002), effectively inhibiting methanogenesis.

At the time of sampling during the drought year, CH_4 emissions had already dropped to a new recent minimum, and the shifting site conditions were evident in the geochemical data. During drought we measured (mostly in the dry subsites) significantly lower dissolved CH_4 concentrations, high dissolved CO_2/CH_4 ratios, as well as a $\delta^{13}C$-CO_2 values around -20‰ in most depth sections (Table 1). High CO_2/CH_4 ratios indicate CO_2 formation from other metabolic processes such as aerobic decomposition, reduction of alternative TEAs, or methane oxidation (Corbett et al., 2013; Holmes et al., 2015; Gao et al., 2019), while CO_2/CH_4 ratios close to one are associated with predominantly methanogenic conditions (Keller et al., 2009). CO_2 produced from non-methanogenic pathways should have a $\delta^{13}C$-CO_2 signature closer to the parent organic matter, while CO_2 produced via methanogenesis is enriched in ^{13}C (Boehme et al., 1996 ; Corbett et al., 2013). Except for two samples, in this study, the $\delta^{13}C$-CO_2 values measured in the dry subsites (ranging -21.6 to -19.6‰) approached -27‰, the average value for C3 plants (Meyers 1994) which are dominant at both sites. Signatures from the wet subsites were typically more enriched in $\delta^{13}C$, with values approaching -10‰ (Table 1). With respect to the measured $\delta^{13}C$-CO^2 values, contribution of older CO_2 and CO_2 formed at depth cannot be excluded. Nevertheless, together, the geochemical data show a shift in redox conditions and associated dominating metabolic pathways, and underscore the differing (more aerobic) site conditions under drought. This is supported by our taxonomic dataset that shows a shift toward the dominance of aerobic bacteria.

Total microbial abundances and community structure

Differences in gene abundances and microbial community structure between the drought and non-drought years were site specific. We expected to detect a lower total abundance of methanogens in both fens due to lowering of the water table and potential associated oxidative stress, as has been documented in previous studies of fen peat (Tian et al., 2012; Tian et al., 2015; Peltoniemi et al., 2016). In this study, total methanogen abundance was significantly lower following drought compared to a non-drought year in the riparian fen (Zarnekow), with no difference in the coastal fen (Hütelmoor), while methanotroph abundance was significantly higher in the coastal fen. In the riparian fen, total methanotroph abundance was greater in only the dry subsites sampled. This observation is likely due to differing site conditions of the fens, differing initial microbial community compositions, and/or stress history of the microbial communities within them. In a mesocosm study, Kim et al., (2008) found short-term drought treatments decreased methanogen abundance in bog, but not in fen peats. Based on this and other evidence, they concluded that distinct microbial communities associated with different peatland types would respond differently to drought effects. Other studies echo this conclusion (Jaatinen et al., 2007; Ho et al., 2016b; Peltoniemi et al., 2016).

Environmental stress history is important in shaping microbial communities, as stress selects for taxa that are able to withstand disturbances (Ho et al., 2016b; Krause et al., 2018; van Kruistum et al., 2018). Although both fens in this study were historically drained for agricultural purposes, microbial communities in the Hütelmoor may be better adapted to environmental stress having experienced fluctuating salinity and water levels associated with historical seawater intrusion. Baumann and Marschner (2013) suggested microorganisms tolerant to salt stress are likely more resistant to desiccation, though the study did not examine CH_4-cycling microbes specifically. Overall, these results show that a months-long, natural drought can change the total abundance of methanogens and methanotrophs, and support previous studies that suggest abundance patterns may differ among individual peatlands.

The ordination analyses further illustrate how patterns in microbial community dynamics may vary among individual peatlands. Microbial community composition differed among the fens, as has often been reported in previous studies (Kim et al., 2008; Peltoniemi et al., 2016; Wen et al., 2018).

In this study, this was true for both the drought and non-drought years. Community composition (including both wet and dry subsites) of bacteria and archaea during drought was distinct from community composition under inundated conditions, with the exception of a few deeper samples that showed some overlap, and of archaea in the Hütelmoor (Fig. D-3). Based on this and the qPCR results, the archaeal community in Zarnekow appeared to be more strongly affected by the drought than in the Hütelmoor. This is in line with some long-term studies that indicate drought alters bacterial and archaeal community composition (Tian et al., 2012; Tian et al., 2015). Other studies suggest that archaeal community composition may be more resistant to warming and/or drying effects in both the short- and long-term (Kim et al., 2008; Peltoniemi et al., 2016). Although archaea community composition converged strongly in the Hütelmoor in the drought year, there was little separation among the polygons indicating the different time points (Fig. D-3 b), suggesting less change in community composition. It is worthwhile to highlight that, while changes in methanogen and methanotroph abundances and overall microbial community composition differed among the fens, CH_4 emission dynamics were ultimately similar, implying functional redundancy among microbial groups. Peltoniemi et al. (2016) reported a similar observation, when they found CH_4 emission dynamics to be similar among two fens subject to warming and drying treatments, despite differing patterns in microbial control over the emissions.

Methanotroph relative abundances and links to laboratory studies

According to 16S *rRNA* gene sequencing, the increase in the relative abundance of type I aerobic methanotrophs, in particular Methylomonaceae, was striking in both fens (Fig. D-4 a and b). In the Hütelmoor, type I methanotrophs were hardly detectable in the non-drought year (Fig. D-4). Previously, it was believed that type II methanotrophs may be more resistant to drought stress due to formation of desiccation-resistant spores (Whittenbury et al., 1970; Bowman 2006). However, more recent work has shown type I methanotrophs to be unexpectedly resistant to environmental stress, as well as responsive to fluctuations in temperature and water availability (Collet et al., 2015; Ho et al., 2016b), while type II methanotroph populations, though ubiquitous, are relatively stable and do not experience significant changes in population size in comparison. To our knowledge, Peltoniemi et al. (2016) were the first to provide *in situ* evidence of this in fens. They found type I methanotroph abundances were altered by warming and drying treatments, while depth was the only controlling factor over type II methanotrophs. Similarly, in our study, there was little difference in the distribution and relative abundance of type II methanotrophs between the drought and non-drought years.

This study provides the first *in situ* evidence from a natural drought that suggests type I methanotrophs are comparatively adaptive to changes in temperature and water availability, which supports earlier experimental work (Henkel et al., 2001; Ho et al., 2013a; Ho et al., 2013b; Pan et al., 2014; Collet et al., 2015; Ho et a. 2016a; Ho et al., 2016b; Peltoniemi et al., 2016). The dominance of type I methanotrophs during drought was confirmed by cDNA sequencing. The type I methanotrophs of the Methylomonaceae family had the highest relative amount of cDNA transcripts (Fig. D-4), and were one of the most abundant bacterial groups (Supplemental data, Fig. D-S2). Thus, type I methanotrophs may represent a significant microbial control over CH_4 emissions in restored temperate fens subject to natural drought.

Evidence for anaerobic methanotrophic activity

Denitrifying anaerobic methanotrophs "*Ca*. M. nitroreducens" (archaea) and "*Ca*. Methylomirabilis" (bacteria) were detected in both the DNA- and cDNA-based community profiles at the same depths. Interestingly, at these depths, the highest $\delta^{13}C-CH_4$ values were measured (20–30 and 40–50 cm depths), and higher $\delta^{13}C$ values (approaching -50‰) in dissolved CH_4 may be indicative of CH_4 oxidation. While this is not conclusive evidence these organisms contribute to CH_4 emissions reduction in these systems, they appear to be active at depth and may represent an additional filter for methane produced in deeper peats. "*Ca*. M. nitroreducens" are known to oxidize significant amounts of methane in rice paddies (Vaksmaa et al., 2017), and rice paddies have been suggested as ideal habitat niches for denitrifying anaerobic methanotrophs because of increased nitrogen availability. Decades of agricultural use (and therefore increased nitrogen availability) during drainage could have promoted the establishment of these organisms at the sites. However, the role of ANME in terrestrial peatlands, however, is little understood and warrants further investigation.

Conclusions

This study shows that reduced CH_4 emissions in drought-affected fens are associated with multiple substantial changes in pore water chemistry and microbial community dynamics, and that some changes in microbial community dynamics may be site specific. In the riparian fen, total methanogen abundance decreased and methanotroph abundance increased in some areas, while, in the coastal fen, only total methanotroph abundances increased. Reduced CH_4 emissions in restored fens subject to natural drought are thus at least partially the result of the differential controlling patterns of methanogens and methanotrophs. However, a large increase in the relative abundance of methanotrophs, particularly type I methanotrophs, was documented in both fens. We provide the first *in situ* evidence from a natural drought that suggests type I methanotrophs are comparably more adaptive than type II methanotrophs when experiencing drought effects, and suggest type I methanotrophs as an important microbial control over methane emissions in temperate fens experiencing natural drought.

Acknowledgements

This work was funded by the DFG (Deutsche Forschungsgemeinschaft) within the framework of the Research Training Group "Baltic TRANSCOAST" (GRK 2000/1). This is Baltic TRANSCOAST publication no. GRK2000/002X. This study was supported by the Helmholtz Gemeinschaft (HGF) by funding the Helmholtz Young Investigators Group of Susanne Liebner (VH-NG-919) and Torsten Sachs (grant VH-NG-821), and by the Terrestrial Environmental Observatories (TERENO) Network. The study was further funded by the European Social Fund (ESF) and the Ministry of Education, Science, and Culture of Mecklenburg-Western Pomerania within the scope of the project WETSCAPES (grants ESF/14-BM-A55-0030/16 and ESF/14-BM-A55-0031/16). We thank Oliver Burckhardt, Anke Saborowski, and Victoria Tietz for their support in the lab. We thank J. Axel Kitte for aiding in both field and lab work. We thank Christian Wille for his work maintaining the Zarnekow eddy covariance tower and processing the raw data. We thank Joachim Hofman for maintaining the Hütelmoor eddy covariance tower.

References

Augustin J., Chojnicki, B. (2008). Austausch von klimarelevanten Spurengasen, Klimawirkung und Kohlenstoffdynamik in den ersten Jahrennach der Wiedervernässung von degradiertem Niedermoorgrünland (Exchange of climate relevant trace gases, climate effect and carbon dynamics in the first years after re-wetting of degraded fen grassland). In: Gelbrecht, J., Zak, D. & Augustin, J. (eds.) Phosphor- und KohlenstoffDynamik und Vegetationsentwicklung in wiedervernässten Mooren des Peenetals in Mecklenburg-Vorpommern (Phosphorus and carbon dynamics and vegetation development in re-wetted peatland of the Peene valley in Mecklenburg-Western Pomerania). Leibniz-Institut für Gewässerökologie und Binnenfischerei, Berlin, 50–67 (in German).

Baird A. J., Beckwith C. W., Waldron S., Waddington, J. M. (2004). Ebullition of methane containing gas bubbles from near-surface Sphagnum peat. Geophysical Research Letters, 31, L21505. doi: 10.1029/2004GL021157

Bastviken D., Tranvik L. J., Downing J. A., Crill P. M., Enrich-Prast A., (2011). Freshwater methane emissions offset the continental carbon sink. Science, 331(6013), 50. doi: 10.1126/science.1196808

Baumann K., Marschner P. (2013). Effects of salinity on microbial tolerance to drying and rewetting. Biogeochemistry, 112, 71-80. doi: 10.1007/s10533-011-9672-1

Bodelier P. L. E., Bär-Gilissen M.-J., Meima-Franke M., Hordijk K. (2012). Structural and functional response of methane-consuming microbial communities to different flooding regimes in riparian soils. Ecology and Evolution, 2(1), 106-127. doi: 10.1002/ece3.34

Boehme S. E., Blair N. E., Chanton J. P., Martens C. S. (1996). A mass balance of ^{13}C and ^{12}C in an organic-rich methane-producing marine sediment. Geochimica et Cosmochimica Acta, 60(20), 3835-3848. doi: 10.1016/0016-7037(96)00204-9

Bolyen E., Rideout J. R., Dillon M. R., Bokulich N. A., Abnet C., Al-Ghalith G. A., Alexander H., Alm E. J., Arumugam M., Asnicar F., Bai Y., Bisanz J. E., Bittinger K., Brejnrod A., Brislawn C. J., Brown C. T., Callahan B. J., Caraballo-Rodríguez A. M., Chase J., Cope E., Da Silva R., Dorrestein P. C., Douglas G. M., Durall D. M., Duvallet C., Edwardson C. F., Ernst M., et al., (2018). QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. PeerJ Preprints, 6 :e27295v2. doi: 10.7287/peerj.preprints.27295v2

Bowman J. P. (2006) The methanotrophs - the families Methylococcaceae and Methylocystaceae. PRO 5:266–289

Callahan B., McMurdie P. J., Rosen M., Han A. W., Johnson A. J. A., Holmes S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13, 581-583. doi: 10.1038/nmeth.3869

Cadillo-Quiroz H., Yashiro E., Yavitt J. B., Stephen H., Zinder S. H. (2008). Characterization of the Archaeal Community in a Minerotrophic Fen and Terminal Restriction Fragment Length Polymorphism-Directed Isolation of a Novel Hydrogenotrophic Methanogen. Applied and Environmental Microbiology, 74(4), 2059-2068. doi: 10.1128/AEM.02222-07

Clark M. G., Humphreys E. R., Carey S. K. (2020). Low methane emissions from a boreal wetland constructed on oil sand mine tailings. Biogeosciences, 17, 667–682, doi: 10.5194/bg-17-667-2020

Collet S., Reim A., Ho A., Frenzel P. (2015). Recovery of paddy soil methanotrophs from long term drought. Soil Biology & Biochemistry, 88, 69-72. doi: 10.1016/j.soilbio.2015.04.016

Corbett J. E., Tfaily M. M., Burdige D.J., Cooper W. T., Glaser P. H., Chanton J. P. (2013). Partitioning pathways of CO_2 production in peatlands with stable carbon isotopes. Biogeochemistry, 114, 327-340. doi: 10.1007/s 10533-012-9813-1

De Vleeschouwer F., Chambers F.M., Swindles G.T. (2010). Coring and sub-sampling of peatlands for palaeoenvironmental research. Mires and Peat, 7: 1, 1-10.

Deutscher Wetterdienst. (2020) Climate and Environment Monthly Description. retrieved on May 26, 2020 from https://www.dwd.de/EN/ourservices/klimakartendeutschland/klimakartendeutschland_monatsbericht.html?nn=519080

Dinsmore K. J.; Skiba U. M., Billett M. F., Rees R. M. (2009). Effect of water table on greenhouse gas emissions from peatland mesocosms. Plant and Soil, 318(1-2), 229-242. doi: 10.1007/s11104-008-9832-9

Dowrick D. J., Freeman C., Lock M. A., Reynolds B. (2006). Sulphate reduction and the suppression of peatland methane emissions following summer drought. Geoderma, 132(3-4), 384-390. doi: 10.1016/j.geoderma.2005.06.003

Estop-Aragonés C., Knorr K.-H., Blodau, C. (2013). Belowground in situ redox dynamics and methanogenesis recovery in a degraded fen during dry-wet cycles and flooding. Biogeosciences, 10, 421–436. doi: 10.5194/bg-10-421-2013, 2013.

Franz D., Koebsch F., Larmanou E., Augustin J., Sachs T. (2016). High net CO_2 and CH_4 release at a eutrophic shallow lake on a formerly drained fen. Biogeosciences, 13, 3051-3070. doi: 10.5194/bg-13-3051-2016

Froelich P. N., Klinkhammer G. P., Bender M. L., Luedtke N. A., Heath G. R., Cullen D., Dauphin P., Hammond D., Hartman B., Maynard V. (1979). Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. Geochimica et Cosmochimica Acta 43, 1075-1090. doi: 10.1016/0016-7037(79)90095-4

Gao C., Sander M. Agethen S. Knorr K.-H. (2019) Electron accepting capacity of dissolved and particulate organic matter control CO_2 and CH_4 formation in peat soils. Geochimica et Cosmochimica Acta, 245, 266-277. doi: 10.1016/j.gca.2018.11.004

Gorham E. (1991). Northern Peatlands: Role in the Carbon Cycle and Probable Responses to Climatic Warming. Ecological Applications 1(2), 182-195. doi: 10.2307/1941811

Hahn J., Köhler S., Glatzel S., Jurasinski G. (2015). Methane Exchange in a Coastal Fen in the First Year after Flooding - A Systems Shift. PLOS ONE 10(10): e0140657. doi: 10.1371/journal.pone.0140657

Hanel M., Rakovec O., Markonis Y., Máca P., Samaniego L., Kyselý J., Kumar, R. (2018) Revisiting the recent European droughts from a long-term perspective. Scientific Reports, 8: 9499. doi: 10.1038/s41598-018-27464-4

Henckel T., Jäckel U., Conrad R. (2001). Vertical distribution of the methanotrophic community after drainage of rice field soil. FEMS Microbiology Ecology, 34, 279-291. doi: 10.1111/j.1574-6941.2001.tb00778.x.

Herlemann D. P., Labrenz M., Jürgens K., Bertilsson S., Waniek J. J., Andersson A. F. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea, ISME J., 5, 1571–1579, doi: 10.1038/ismej.2011.41

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

Ho A., Luke C., Reim A., Frenzel P. (2013b). Selective stimulation in a natural community of methane oxidizing bacteria: effects of copper on *pmoA* transcription and activity. Soil Biology & Biochemistry, 65, 211-216. doi: 10.1016/j.soilbio.2013.05.027

Ho A., van den Brink E., Reim A., Krause S. M. B., Bodelier P. L. E. (2016a). Recurrence and Frequency of Disturbance have Cumulative Effect on Methanotrophic Activity, Abundance, and Community Structure. Frontiers in Microbiology, 6:1493. doi: 10.3389/fmicb.2015.01493

Ho A., Lüke C., Reim A., Frenzel P. (2016b). Resilience of (seed bank) aerobic methanotrophs and methanotrophic activity to desiccation and heat stress. Soil Biology & Biochemistry, 101, 130-138. doi: 10.1016/j.soilbio.2016.07.015

Holmes M. E., Chanton J. P., Tfaily M. M., Ogram A. (2015). CO_2 and CH_4 isotope compositions and production pathways in a tropical peatland. Global Biogeochemical Cycles, 29, 1-18. doi: 10.1002/2014GB004951

IPCC. (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Jaatinen K., Fritze H., Laine J., Laiho R. (2007). Effects of short- and long-term water-level drawdown on the populations and activity of aerobic decomposers in a boreal peatland. Global Change Biology, 13(2), 491-510. doi: 10.1111/j.1365-2486.2006.01312.x

Jasso-Chávez R., Santiago-Martínez M. G., Lira-Silva E., Pineda E., Zepeda-Rodríguez, A., Belmont-Díaz J., Encalada R., Saavedra E., Moreno-Sánchez R. (2015). Air-Adapted Methanosarcina acetivorans Shows High Methane Production and Develops Resistance against Oxygen Stress. PLoS ONE 10(2), e0117331. doi : 10.1371/journal.pone.0117331

Joabsson A., Christensen T.R., Wallén, B. (1999). Vascular plant controls on methane emissions from northern peatforming wetlands. Trends in Ecology and Evolution, 14(10), 385–388. doi: 10.1016/s0169-5347(99)01649-3.

Kang X., Yan L., Cui L., Zhang X., Hao Y., Wu H., Zhang Y., Li W., Zhang K., Yan Z., Li Y., Wang J. (2018). Reduced Carbon Dioxide Sink and Methane Source under Extreme Drought Condition in an Alpine Peatland. Sustainability, 10, 4285. doi: 10.3390/su10114285

Keller J. K., Weisenhorn P. B., Megonigal J. P. (2009). Humic acids as electron acceptors in wetland decomposition. Soil Biology & Biochemistry, 41(7), 1518-1522. doi: 10.1016/j.soilbio.2009.04.008

Kim S.-Y., Lee S.-H. Freeman C., Fenner N., Kang H. (2008). Comparative analysis of soil microbial communities and their responses to the short-term drought in bog, fen, and riparian wetlands. Soil Biology & Biochemistry, 40, 2874-2880. doi: 10.1016/j.soilbio.2008.08.004

Knief C. (2015). Diversity and Habitat Preferences of Cultivated and Uncultivated Aerobic Methanotrophic Bacteria Evaluated Based on *pmoA* as Molecular Marker. Frontiers in Microbiology, 6: 1346. doi: 10.3389/fmicb.2015.01346

Knorr K.-H., Oosterwoud M. R., Blodau C. (2008). Experimental drought alters rates of soil respiration and methanogenesis but not carbon exchange in soil of a temperate fen. Soil Biology & Biochemistry, 40, 1781-1791. doi: 10.1016/j.soilbio.2008.03.019

Knorr K.-H., Blodau, C. (2009). Impact of experimental drought and rewetting on redox transformations and methanogenesis in mesocosms of a northern fen soil. Soil Biology & Biochemistry, 41(6), 1187-1198. doi:10.1016/j.soilbio.2009.02.030

Koebsch F., Winkel M., Liebner S., Liu B., Westphal J., Schmiedinger I., Spitzy A., Gehre M., Jurasinski G., Köhler S., Unger V., Koch M., Sachs T., Böttcher M.E. (2019). Sulfate deprivation triggers high methane production in a disturbed and rewetted coastal peatland. Biogeosciences, 16, 1937-1953. doi: 10.5194/bg-16-1937-2019

Krause S. M. B., Meima-Franke M., Veraart A. J., Ren G., Ho A., Bodelier P. L. E. (2018). Environmental legacy contributes to the resilience of methane consumption in a laboratory microcosm system. Scientific Reports, 8: 8862. doi: 10.1038/s41598-018-27168-9

Kristjansson J. K., Schönheit, P. (1983). Why do sulfate-reducing bacteria outcompete methanogenic bacteria for substrates? Oecologia, 60, 264-266. doi: 10.1007/BF00379530

Laiho R. (2006). Decomposition in peatlands: Reconciling seemingly contrasting results on the impacts of lowered water levels. Soil Biology & Biochemistry, 38(8), 2011-2024. doi: 10.1016/j.soilbio.2006.02.017

Liebner S., Svenning M. M. (2013). Environmental Transcription of mmoX by Methane-Oxidizing Proteobacteria in a Subarctic Palsa Peatland. Applied and Environmental Microbiology, 79, 2, 701-706. doi: 10.1128/AEM.02292-12.

Ma K., Lu Y. (2011). Regulation of microbial methane production and oxidation by intermittent drainage in rice field soil. FEMS Microbiology Ecology, 75(3), 446-456. doi: 10.1111/j.1574-6941.2010.01018.x

Ma K., Conrad R., Lu Y. (2013). Dry/Wet Cycles Change the Activity and Population Dynamics of Methanotrophs in Rice Field Soil. Applied and Environmental Biology, 79(16), 4932-4939. doi: 10.1128/AEM.00850-13

Magnusson L., Ferranti L., Vamborg F. (2018). Forecasting the 2018 European heatwave. ECMWF Newsletter, 157, 4.

Manheim F.T. (1966). A hydraulic squeezer for obtaining interstitial water from consolidated and unconsolidated sediments. USGS Ref. Pap. 550, 171–174.

Martin M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads, EMBnet. Journal, 17, 10–12. doi: 10.14806/ej.17.1.200, 2011.

Meyers P.A. (1994). Preservation of elemental and isotopic source identification of sedimentary organic matter. Chemical Geology, 114, 289-302. doi: 10.1016/0009-2541(94)90059-0

Moore T., Roulet N., Knowles R. (1990). Spatial and temporal variations of methane flux from subarctic/northern boreal fens. Global Biogeochemical Cycle, 4(1), 29-46. doi: 10.1029/GB004i001p00029

Morozova D., Wagner D. (2007). Stress response of methanogenic archaea from Siberian permafrost compared with methanogens from nonpermafrost habitats. FEMS Microbiology Ecology, 61(1), 16-25. doi: 10.1111/j.1574-6941.2007.00316.x

NOAA National Centers for Environmental Information, State of the Climate: Global Climate Report for April 2020, published online May 2020, retrieved on May 26, 2020 from https://www.ncdc.noaa.gov/sotc/global/202004.

Oksanen J., Blanchet F. G., Friendly M., Kindt R., Legendre P., McGlinn D., Minchin P. R., O'Hara R. B., Simpson G. L., Solymos P., Stevens M. H. H., Szoecs E., and Wagner H. (2019).

vegan: Community Ecology Package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan

Pan Y., Abell G. C. J., Bodelier P. L. E., Meima-Franke M., Sessitsch A., Bodrossy L. (2014). R emarkable recovery and colonization behavior of methane oxidizing bacteria in soil after disturbance is controlled by methane course only. Microbial Ecology, 68, 259-270. doi: 10.1007/s00248-014-0402-9

Peltoniemi K., Laiho R., Juottonen H., Bodrossy L., Kell D.K., Minkkinen K., Mäkiranta P., Mehtätalo L., Penttilä T., Siljanen H.M.P., Tuittila E.-S., Tuomivirta T., 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. doi: 10.1016/j.soilbio.2016.03.007

Peters V., Conrad R. (1996). Sequential reduction processes and initiation of CH_4 production upon flooding of oxic upland soils. Soil Biology & Biochemistry, 28(3), 371-382. doi: 10.1016/0038-0717(95)00146-8

Potter C., Freeman C., Golyshin P. N., Ackermann G., Fenner N., McDonald J. E., Ehbair A., Jones T. G., Murphy L. M., Creer S. (2017). Subtle shifts in microbial communities occur alongside the release of carbon induced by drought and rewetting in contrasting peatland ecosystems. Scientific Reports, 7: 11314. doi: 10.1038/s41598-017-11546-w

Quast C., Pruesse E., Yilmaz P., Gerken J., Schweer T., Yarza P., Peplies J., Glöckner F.O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, Nucleic Acids Research, 41, D590–596, doi: 10.1093/nar/gks1219

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rodem E. E., Wetzel R.G. (1996). Organic carbon oxidation and suppression of methane production by microbial Fe(III) oxide reduction in vegetated and unvegetated freshwater wetland sediments. Limnology and Oceanography, 41(8), 1733-1748.

Rognes T., Flouri T., Nichols B., Quince C., Mahé F. (2016). VSEARCH: a versatile open source tool for metagenomics. PeerJ, 4: e2584. doi: 10.7717/peerj.2584

Scholten J. C. M., van Bodegom P.M., Vogelaar J., van Ittersum A., Roelofsen W., Stams A. J. M. Effect of sulfate and nitrate on acetate conversion by anaerobic microorganisms in a freshwater sediment. FEMS Microbiology Ecology, 42(3), 375-385. doi: 10.1111/j.1574-6941.2002.tb01027.x

Tian J., Zhu Y., Kang X., Dong X., Li W., Chen H., Wang Y. (2012). Effects of drought on the archaeal community in soil of the Zoige wetlands of the Qinghai–Tibetan plateau. European Journal of Soil biology, 52, 84-90. doi: 10.1016/j.ejsobi.2012.07.003

Tian J., Shu C., Chen H., Qiao Y., Yang G., Xiong W., Wang L., Sun J., Liu X. (2015). Response of archaeal communities to water regimes under simulated warming and drought conditions in Tibetan Plateau wetlands. Journal of Soils and Sediments, 15, 179-188. doi: 10.1007/s11368-014-0978-1

Takai K., Horikoshi K. (2000). Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. Applied and Environmental Microbiology, 66, 5066–5072. doi: 10.1128/AEM.66.11.5066-5072.2000

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: G00A10. doi:10.1029/2007JG000496

van Kruistum H., Bodelier P. L. E., Ho A., Meima-Franke M., Veraart A.J. (2018). Resistance and Recovery of Methane-Oxidizing Communities Depends on Stress Regime and History; A Microcosm Study. Frontiers in Microbiology, 9: 1714. doi: 10.3389/fmicb.2018.01714

Wen X., Unger V., Jurasinski G., Koebsch F., Horn F., Rehder G., Sachs T., Zak D., Lischeid G., Knorr K.-H., Böttcher M. E., Winkel M., Bodelier P. L. E., Liebner S. (2018). Predominance of methanogens over methanotrophs in rewetted fens characterized by high methane emissions. Biogeosciences, 15, 6519-6536. doi: 10.5194/bg-15-6519-2018

Whittenbury R., Davies S. L., Davey J. F. (1970). Exospores and cysts formed by methane-utilizing bacteria. Journal of General Microbiology, 61, 219-226. doi: 10.1099/00221287-61-2-219

Yavitt J. B., Lang G. E., Downey D. M. (1988). Potential methane production and methane oxidation rates in peatland ecosystems of the Appalachian Mountains, United States. Global Biogeochemical Cycles, 2(3), 253-268.

Yu Z., Beilman D. W., Frolking S., MacDonald G. M., Roulet N. T., Camill P., Charman D. J. (2011). Peatlands and their role in the global carbon cycle. Eos, 92(12), 97-98. doi: 10.1029/2011EO120001

Whittenbury, R., Davies, S. L., and Davey, J. (1970). Exospores and cysts formed by methane-utilizing bacteria. Microbiology, 61, 219–226. doi: 10.1099/00221287-61-2-219

Zehnder A. J. B., Stumm W. (1988). Geochemistry and Biogeochemistry of anaerobic habitats. In: A. J. B. Zehnder (Ed.), Wiley series in ecological and applied microbiology. Biology of anaerobic microorganisms (pp. 1-38). New York: Wiley.

Supplement

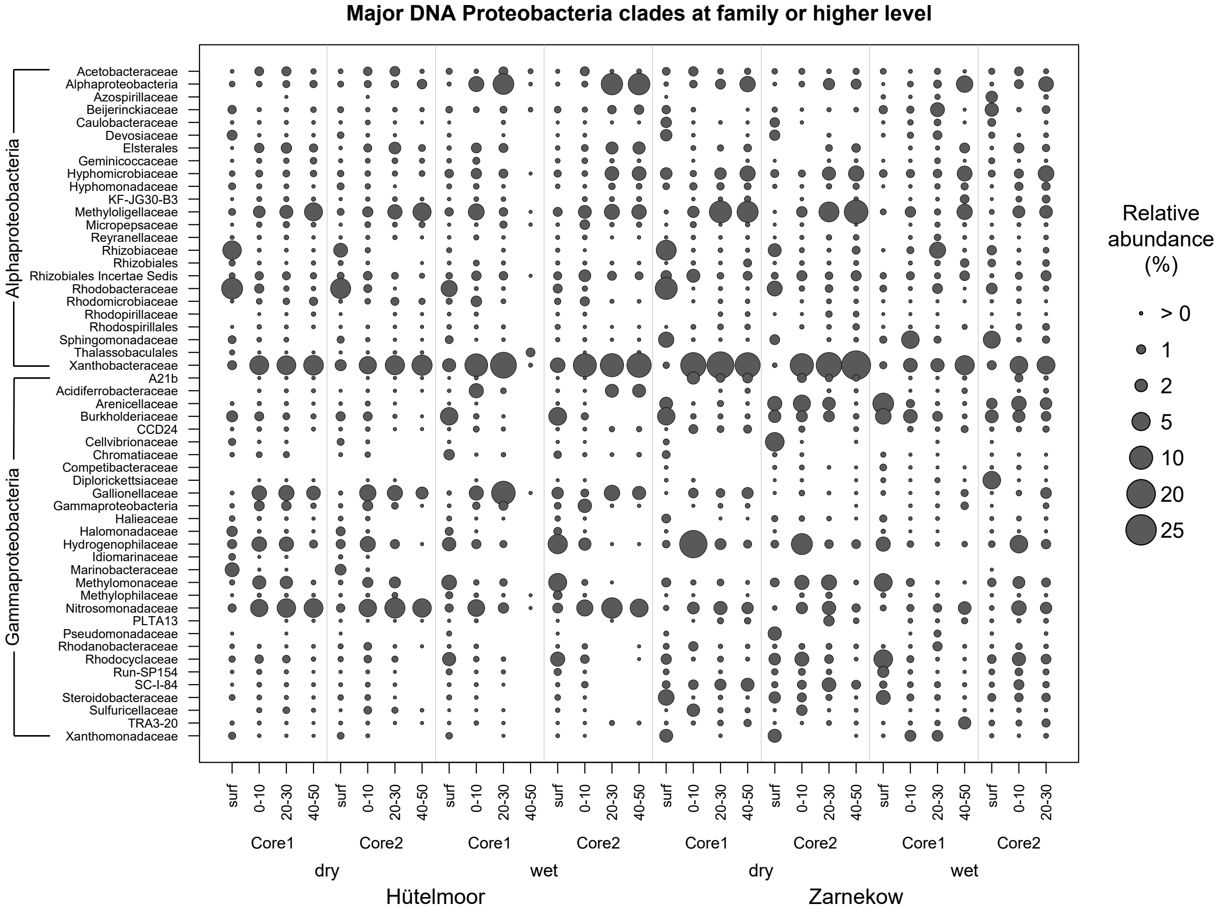


Figure D-S1 – Bubble plots showing relative abundance of major bacterial families during the 2018 summer drought based on 16S rRNA gene sequencing. Samples are arranged along the x-axis according to site (HM and ZN for the Hütelmoor and Zarnekow, respectively) and depth section in cm (surf = surface samples) given as a range. The Abbreviations DU and WU denote samples from dry unvegetated and wet unvegetated subsites, respectively. Taxa were placed at the next possible higher taxonomic level if assignment to the family level was not possible.

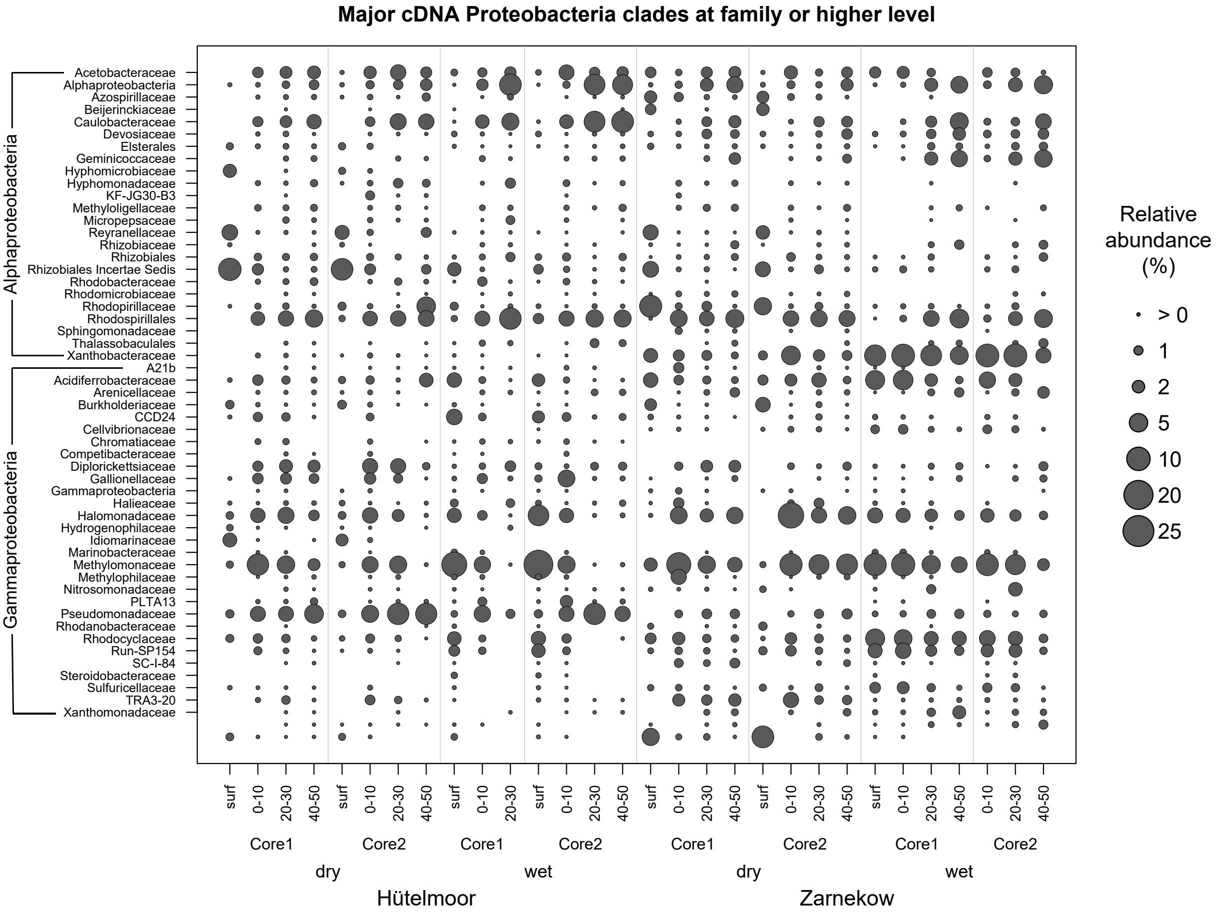


Figure D-S2 – Bubble plots showing relative abundance of active major bacterial families during the 2018 summer drought based on cDNA sequencing. Samples are arranged along the x-axis according to site (HM and ZN for the Hütelmoor and Zarnekow, respectively) and depth section in cm (surf = surface samples) given as a range. The Abbreviations DU and WU denote samples from dry unvegetated and wet unvegetated subsites, respectively. Taxa were placed at the next possible higher taxonomic level if assignment to the family level was not possible.

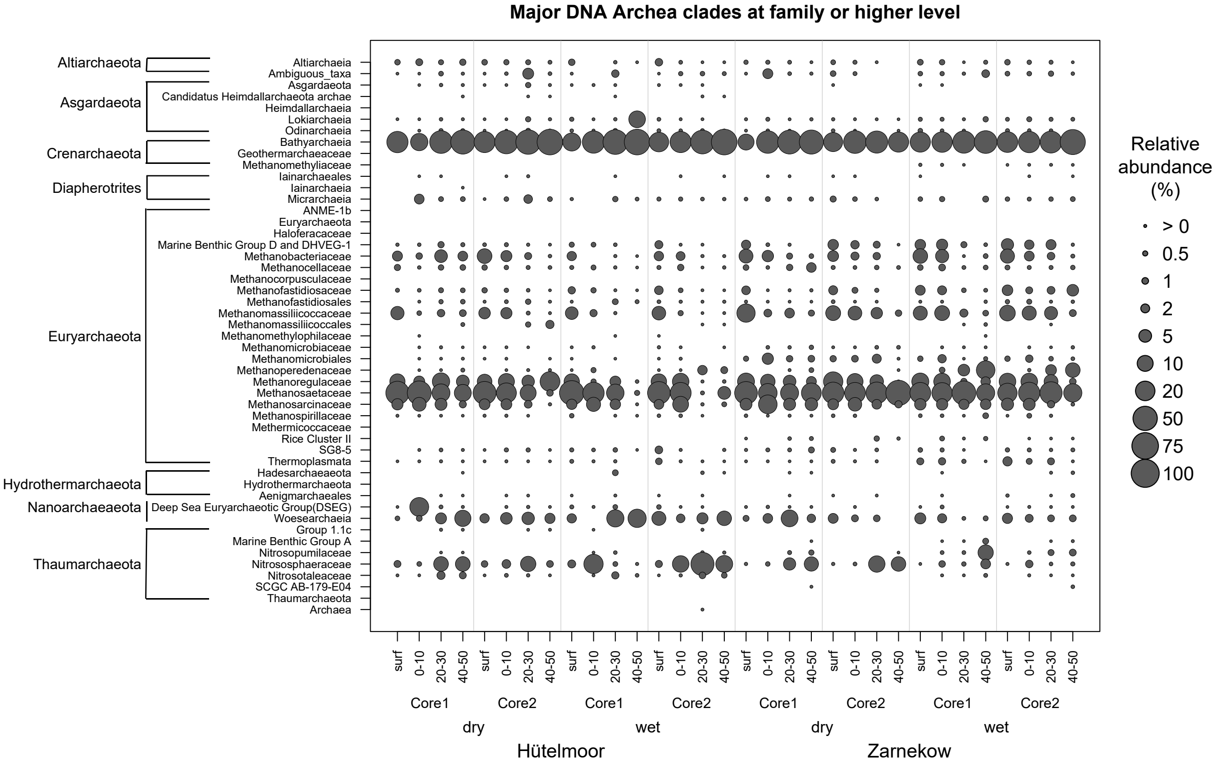


Figure D-S3 – Bubble plots showing relative abundance of major archaeal families during the 2018 summer drought based on 16S rRNA gene sequencing. Samples are arranged along the x-axis according to site (HM and ZN for the Hütelmoor and Zarnekow, respectively) and depth section in cm (surf = surface samples) given as a range. The Abbreviations DU and WU denote samples from dry unvegetated and wet unvegetated subsites, respectively. Taxa were placed at the next possible higher taxonomic level if assignment to the family level was not possible.

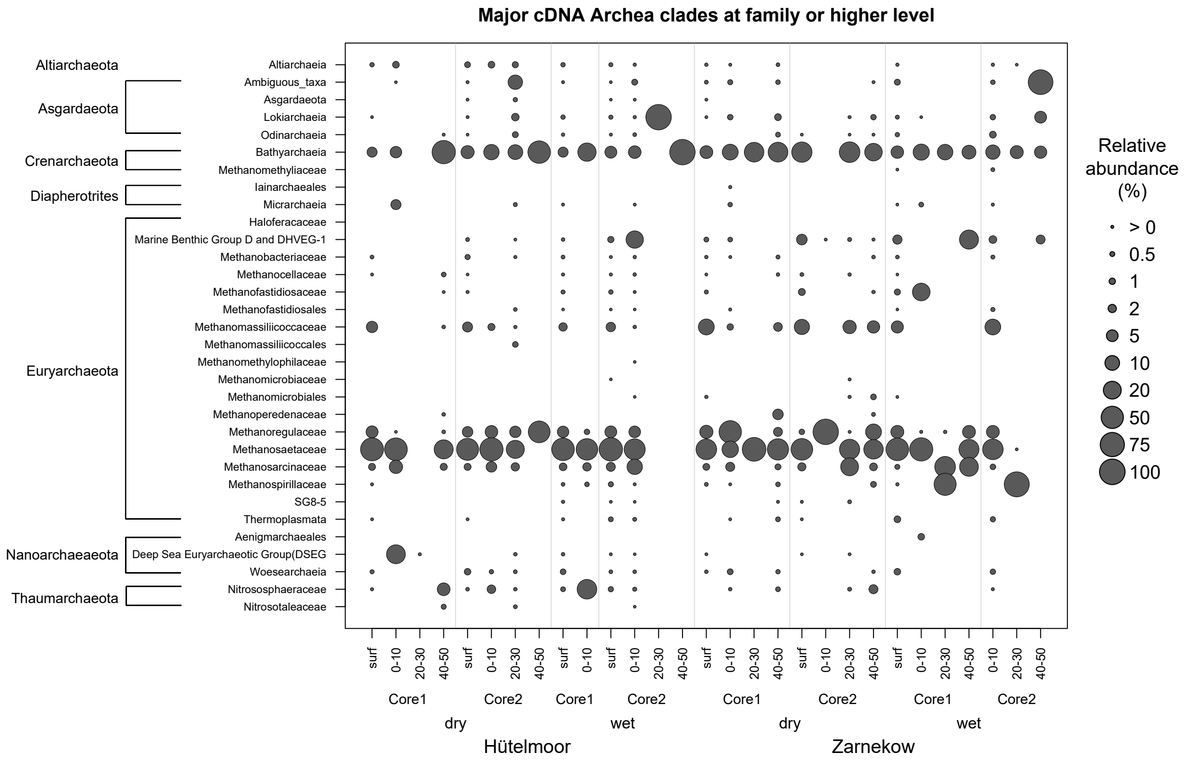


Figure D-S4 – Bubble plots showing relative abundance of active major archaeal families during the 2018 summer drought based on cDNA sequencing. Samples are arranged along the x-axis according to site (HM and ZN for the Hütelmoor and Zarnekow, respectively) and depth

Curriculum Vitae

Personal information

Name:	Viktoria Unger
Born:	04.04.1989 in Philadelphia, PA, USA
Nationality:	Dual - US American, German
Languages:	English (native speaker), German (B.1 level)

Professional employment and education

Since Jan 2016	PhD candidate, Landscape Ecology and Site Evaluation, Faculty of Agricultural and Environmental Science, Rostock University, Germany (Prof. G. Jurasinski), *Expected graduation May 2021*
Sep 2013–Jun 2014	Adjunct Professor, Department of Earth and Environmental Science, Temple University, Philadelphia, PA, USA Adjunct Professor, College of Science, Health, and Liberal Arts, Philadelphia University (Now Thomas Jefferson University East Falls campus), Philadelphia, PA, USA Research Assistant, Academy of Natural Sciences Ruth Patrick Center for Environmental Research, Philadelphia, PA, USA
Sep 2011–Aug 2013	MSc Student: Environmental Science, Drexel University, Philadelphia, USA (Prof. T. Elsey-Quirk, Prof. S. Kilham), Thesis title: Carbon accumulation in salt marsh soils: a comparison of a coastal plain and coastal lagoon estuary in the mid-Atlantic U.S., *GPA: 3.7* Research Assistant, Academy of Natural Sciences Ruth Patrick Center for Environmental Research, Philadelphia, PA, USA
Sep 2007–Aug 2011	BSc student: Environmental and Conservation Biology, Thomas Jefferson University East Falls Campus, Philadelphia, PA, USA, *GPA 3.3* Teaching assistant for Biology I, II and Chemistry I, II

Publications (V. Unger as corresponding or lead author **in bold type**)

Zak, D., Roth, C., Unger, V., Goldhammer, T., Fenner, N., Freeman, C., and Jurasinski, G. (2019) Unravelling the importance of polyphenols for microbial carbon mineralization in rewetted riparian peatlands. *Front. Environ. Sci.* DOI: 10.3389/fenvs.2019.00147

Koebsch, F., Winkel, M., Liebner, S., Liu, B., Westphal, J., Schmiedinger, I., Spitzy, A., Gehre, M., Jurasinski, G., Köhler, S., Unger, V., Koch, M., Sachs, T., and Böttcher, M. E. (2019) Sulfate deprivation triggers high methane production in a disturbed and rewetted coastal peatland. *Biogeosciences* 16: 1937-1953. DOI: 10.5194/bg-16-1937-2019

Wen, X.*, **Unger, V.**, Jurasinski, G., Koebsch, F., Horn, F., Rehder, G., Sachs, T., Zak, D., Lischeid, G., Knorr, K-H., Böttcher, M. E., Winkel, M., Bodelier, P. L. E., and Liebner, S. (2018) Predominance of methanogens over methanotrophs in rewetted fens characterized by high methane emissions. *Biogeosciences* 15: 6519-6536. DOI: 10.5194/bg-15-6519-2018

- ➢ shared first authorship

Jurasinski, G., Janssen, M., Voss, M., Böttcher, M. E., Brede, M., Burchard, H., Forster, S., Gosch, L., Gräwe, U., Gründling-Pfaff, S., Haider, F., Ibenthal, M., Karow, N., Karsten, U., Kreuzburg, M., Lange, X., Leinweber, P., Massmann, G., Ptak, T., Rezanezhad, F., Rehder, G., Romoth, K., Schade, H., Schubert, H., Schulz-Vogt, H., Sokolova, I. M., Strehse, R., Unger, V., Westphal, J., Lennartz, B. (2018) Understanding the Coastal Ecocline: Assessing Sea–Land Interactions at Non-tidal, Low-Lying Coasts Through Interdisciplinary Research
Front. Mar. Sci. DOI: 10.3389/fmars.2018.00342

Elsey-Quirk, T. and Unger, V. (2018) Geomorphic influences on the contribution of vegetation to soil C accumulation and accretion in Spartina alterniflora marshes. *Biogeosciences* 15(1):379-397. DOI: 10.5194/bg-15-379-2018

Unger. V., Elsey-Quirk, T., Sommerfield, C., and Velinsky, D. J. (2016) Stability of organic carbon accumulating in Spartina alterniflora-dominated salt marshes of the Mid-Atlantic U.S. Estuarine, coastal, and shelf science 182(a): 179-189. DOI: 10.1016/j.ecss.2016.10.001

Submitted manuscripts:

Unger, V., Liebner, S., Koebsch, F., Horn, F., Yang, S., Sachs, T., Kallmeyer, J., Knorr, H-K., Rehder, G., Gottschalk, P., and Jurasinski, G. (*in review, SBB*) Congruent changes in microbial community dynamics and ecosystem methane fluxes following natural drought in two restored fens.

Conference contributions

2018 Ecology of Soil Microorganisms symposium, Helsinki, Finland (*oral presentation*) Title: Predominance of methanogens over methanotrophs in rewetted fens characterized by high methane emissions

2018 Ocean Sciences Meeting, Portland, Oregon (*oral presentation*) Title: Production High, Consumption Low – Microbial Evidence for Heightened Methane Emissions in Rewetted Fens

2013 21st Biennial conference of the Coastal and Estuarine Research Federation, San Diego, CA, USA (*oral presentation*) Title: The relationship between carbon burial and sediment deposition in salt marshes: A comparison of a coastal lagoon and coastal plain estuary in the mid-Atlantic U.S.

2013 Delaware Estuary Science and Environmental Summit, Cape May, NJ, USA (*oral presentation*) Title: The relationship between carbon burial and sediment deposition in salt marshes: A comparison of a coastal lagoon and coastal plain estuary in the mid-Atlantic U.S.

Teaching experience

Courses

2014 *Microbiology* (lab and theory), undergraduate level for science majors, Thomas Jefferson University East Falls Campus

Evolution and extinctions (lab and theory), undergraduate level for non-science majors, Temple University

Climate change (lab and theory), undergraduate level for science majors, Temple University

Media and public outreach

2012 Interview, research featured in Drexel University’s DrexelNOW Magazine, Philadelphia, PA, USA

2010 Interview, research featured in Philadelphia University’s Innovator magazine, Philadelphia, PA, USA

2010 Oral presentation to officials of the Royal Government of Bhutan, Rural watershed assessment report, Thimphu, Bhutan

2009 Oral presentation to Philadelphia University’s Board of Trustees, Student sustainability awareness

Theses/Thesen zur Dissertation

Rationale and objectives of the research

The rewetting of drained fens is an often-employed restoration technique intended to inhibit peat greenhouse gas emissions associated with drainage, but rewetting often leads to high methane emissions in temperate minerotrophic fens. The factors that contribute to the magnitude and duration of increased emissions are not well-resolved. Despite them representing a significant control over methane production and emission, peat microbial communities have rarely been investigated in these ecosystems. Studies of microbial community dynamics (mainly, community composition, abundances, and activity) in rewetted fens would provide additional insight into observed patterns in methane emissions in these systems, and aid in making better-informed management decisions regarding rewetting practices. Additionally, climate-change related extreme weather events are expected to increase, with unresolved alterations to fen methane cycling and the involved microbial communities. In order to anticipate potential alterations to fen methane cycling under global change, a thorough understanding of the microbial communities both directly and indirectly involved in methane cycling must be achieved.

The aim of this thesis was to elucidate patterns in microbial community dynamics (namely, community composition, abundances, and activity) in rewetted temperate fens, in the framework of methane cycling. The specific objectives were:

- To identify and enumerate microbial communities involved in methane cycling in temperate minerotrophic fens
- To determine if patterns in the community composition and abundance of methane-cycling microbes reflect observed patterns in methane emissions
- To determine if long-term drainage leads to decreased polyphenol content, higher microbial activity, and potentially higher methane production in rewetted fen peats
- To characterize the community dynamics of methane-cycling microbes during a natural drought

Methods

- DNA extraction and 16S rRNA gene sequencing were employed for detection and taxonomic assignment of peat microbes in two rewtted fens in both a drought and non-drought year. This method also allowed estimation of microbial relative abundances.
- Quantitative polymerase chain reaction (qPCR) was employed to enumerate methanogens and methnotrophs.
- Fluorscein diacetate hydrolysis (FDA) was employed to investigate overall microbial activity in rewetted fen peats with different polyphenol concentrations.

- Total RNA extraction, reverse transcription, and subsequent sequencing of genetic material were employed to identify peat microbial taxa active during a natural drought.

Main results

- High ratios of methanogens (methane-producers) to methanotrophs (methane-consumers) were found to be a characteristic of two rewetted temperate fens (a coastal brackish and a freshwater riparian fen) with high methane emissions, despite differing environmental settings and site geochemistry. The relative abundance of methanotrophs in both fens was significantly lower than published values for pristine fens.

- Rewetted fen peats contain significantly lower amounts of microbe-inhibiting polyphenols. Fen peats with the lowest concentrations of polyphenols had the highest amount of microbial activity as measured with fluorescein diacetate hydrolosis.

- Methanotroph relative abundances were significantly higher in both the coastal brackish and freshwater riparian fen during drought. In the freshwater riparian fen, total methanogen abundances were lower, while in the coastal fen, total methanotroph abundances were higher.

Conclusions and outlook

- The studies in this thesis provide an important first look into the microbial communities important for methane cycling in rewetted fens.

- Patterns in the community composition and abundance of methanogens and methanotrophs reflect the observed ecosystem methane emissions.

- Rewetting via flooding may inhibit methanotroph establishment in the early phases of rewetting. Pulse-rewetting may be an alternative to rewetting via flooding, as it has been linked to lower methane emissions, and could possibly benefit some methanotrophs.

- Long-term drainage may leave a biogeochemical legacy in the form of polyphenol degradation, which could potentially contribute to higher methane production in rewetted fens. Top soil removal could potentially be utilized as a mitigative technique in this regard.

- A months-long water level reduction induced by natural drought may benefit methanotrophs and enhance methane oxidation. At the same time, it can reduce the abundance of methanogens significantly.

Acknowledgements

This thesis was funded by the Deutsche Forschungsgemeinschaft (DFG) within the framework of the research training group "Baltic TRANSCOAST" (GRK 2000/1).

First, I want to thank my main supervisor Dr. Gerald Jurasinski for always believing in me, and remaining positive that we'd conduct great studies, despite all the difficulties. I especially appreciate his help with data analysis, as well as his enthusiasm about the "bigger picture" of what we were doing. This, to me, is what makes research enjoyable.

I'd also like to thank my co-supervisor Dr. Franziska Koebsch, who probably witnessed – and helped me through – the majority of my worst panics. Besides the emotional support, her suggestions never failed to substantially increase the quality of my thought-process and writing.

I'd like to thank my co-supervisor Prof. Dr. Gregor Rehder for helping me establish realistic goals, for his constant encouragement, for always making sure my geochemical analyses were sound, and finally, for instantly forgiving me that one time I forgot to include him on the e-mail with my first paper draft.

And finally, I'd like to thank my last co-supervisor, Jr. Prof. Dr. Susanne Liebner, who took me under her wing when I conducted microbial analyses in her labs. My time at the GFZ was one of the most memorable moments of my career so far, and I learned a great deal about peatland microbial communities thanks to her expertise. Besides this, she included me in many social outings, from which I've gained lasting friendships.

I also need to thank others without whom this never would have been possible. First of all, many thanks to Prof. Dr. Torsten Sachs, who allowed me to stay in his apartment in Potsdam well past my predicted stay-time. I can't emphasize enough how much I enjoyed my time there. Many thanks also to my colleagues and co-students at the AUF. I appreciate the help with all the little challenges along the way, and am grateful for them always remembering me on my birthday, even when I was away in Potsdam. I'd also like to thank the other students and PIs in the Baltic Transcoast project. I am very grateful for the help and advice, and will never forget the fun and interesting trips we took to Sweden and Finland.

I'd like to thank my sister Anne, and my friends back in the USA, especially Janelle, who regularly checked on me and my mental state, and encouraged me to continute despite probably still being salty with me for leaving.

I also want to thank Robert Strehse, who helped me since the day I arrived in Germany, and has been a constant pillar of support since then.

Last, but certainly not least, I want to thank my parents, Marie and Götz Unger, whose relentless support made all of this possible for me to achieve.

Selbständigkeitserklärung

Hiermit erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die dabei angegebenen Quellen und Hilfsmittel benutzt habe.

Rostock, 07.10.2020

Viktoria R. Unger