Helmholtz-Zentrum Potsdam Deutsches GeoForschungsZentrum

Geomikrobiologie

Distribution patterns and environmental drivers of methane-cycling microorganisms in natural environments and restored wetlands

Kumulative Dissertation

zur Erlangung des akademischen Grades

"doctor rerum naturalium"

-Dr. rer. Nat.-

in der Wissenschaftsdisziplin "Bioinformatik" vorgelegt an der

Mathematisch-Naturwissenschaftlichen Fakultät

der Universität Potsdam

von

Xi Wen

Potsdam, Oktober 2019

Supervisor: Prof. Dr. Susanne Liebner

Date of final exam: 09. März 2020

Published online in the
Institutional Repository of the University of Potsdam:
https://doi.org/10.25932/publishup-47177
https://nbn-resolving.org/urn:nbn:de:kobv:517-opus4-471770

Statement of Original Authorship

Herewith, I assure that I have developed and written the enclosed PhD-thesis completely by myself, and have not used sources or means without declaration in the text. Any thoughts from others or literal quotations are clearly marked. The PhD-thesis was not used in the same or in a similar version to achieve an academic grading or is being published elsewhere.

Xi Wen

Preface

This study was funded by the China Scholarship Council (CSC, Grant No. 201408620031) and the Helmholtz Gemeinschaft (HGF) through the Helmholtz Young Investigators Group of Susanne Liebner (VH-NG-919), and further supported by the Terrestrial Environmental Observatories (TERENO) Network. The present work focuses on the methane-cycling microorganisms in natural environments and restored wetlands and provides insights into the biogeographic distribution patterns and their environmental drivers of methane-cycling microbial communities at global and local (wetlands in northeastern Germany) scales.

This study is written in English and is presented as a cumulative PhD thesis at the Institute for Biochemistry and Biology at the Faculty of Mathematics and Natural Science, University of Potsdam.

The thesis is composed of an introduction (chapter 1) and three main chapters (2-4), followed by a synthesis and conclusion (chapter 5). The first chapter gives an introduction of the research background, a short description of the study sites, the main objectives of this work as well as a summary of the publications. The main chapters consist of three manuscripts with first authorship (chapters 2-4) of which one is a shared first authorship (chapter 3). The synthesis of the three publications is given in chapter 5, in which the major conclusions and future perspectives are summarized. In addition, there is a coauthor (2^{nd} authorship) paper, which was listed in the supplement but not included in this thesis.

Acknowledgments

First and foremost, my sincere gratitude goes to my supervisor Prof. Dr. Susanne Liebner. I would like to thank her for providing me the opportunity to enjoy Geomicrobiology at GFZ Potsdam, for all the continuous support and encouragement she gave to me, for her enthusiasm and patience. No matter how frustrating I was, she always helped me to find the bright and positive side.

I gratefully acknowledge my second supervisor Prof. Dr. Dirk Wagner for all his help during my scholarship application, for his guidance and valuable input during my study. I am also grateful to my mentor Dr. Fabian Horn who helped me a lot in statistics and bioinformatics.

Many thanks to all my colleagues of the section of Geomicrobiology for providing me with a very stimulating environment and giving me an enjoyable time at GFZ Potsdam. With a special mention to Dr. Lars Ganzert, Dr. Steffi Genderjahn, Julia Mitzscherling, Dr. Matthias Winkel, Stine Holm, and Andrea Kiss that have taken some time to discuss and enrich my work.

A special gratitude goes to China Scholarship Council (CSC) for helping me and providing funding of my project (Grant No. 201408620031).

I also wish to thank all of my family and friends who have supported me along the journey.

Finally, but no means least, thanks go to my parents for providing me almost unbelievable emotional support in my life. Thanks Sizhong, who has been by my side throughout this challenging period and without him this PhD would not have been achievable. Most special thanks to my darling Mulin and Peilin for being so supportive during the past few years / months and making my life more complete. I love you all dearly.

Summary

Methane is an important greenhouse gas contributing to global climate change. Natural environments and restored wetlands contribute a large proportion to the global methane budget. Methanogenic archaea (methanogens) and methane oxidizing bacteria (methanotrophs), the biogenic producers and consumers of methane, play key roles in the methane cycle in those environments. A large number of studies revealed the distribution, diversity and composition of these microorganisms in individual habitats. However, uncertainties exist in predicting the response and feedback of methane-cycling microorganisms to future climate changes and related environmental changes due to the limited spatial scales considered so far, and due to a poor recognition of the biogeography of these important microorganisms combining global and local scales.

With the aim of improving our understanding about whether and how methane-cycling microbial communities will be affected by a series of dynamic environmental factors in response to climate change, this PhD thesis investigates the biogeographic patterns of methane-cycling communities, and the driving factors which define these patterns at different spatial scales. At the global scale, a meta-analysis was performed by implementing 94 globally distributed public datasets together with environmental data from various natural environments including soils, lake sediments, estuaries, marine sediments, hydrothermal sediments and mud volcanos. In combination with a global biogeographic map of methanogenic archaea from multiple natural environments, this thesis revealed that biogeographic patterns of methanogens exist. The terrestrial habitats showed higher alpha diversities than marine environments. *Methanoculleus* and *Methanosaeta* (*Methanothrix*) are the most frequently detected taxa in marine habitats, while *Methanoregula* prevails in terrestrial habitats. Estuary ecosystems, the transition zones between marine and terrestrial/limnic ecosystems, have the highest methanogenic richness but comparably low methane emission rates.

At the local scale, this study compared two rewetted fens with known high methane emissions in northeastern Germany, a coastal brackish fen (Hütelmoor) and a freshwater riparian fen (Polder Zarnekow). Consistent with different geochemical conditions and land-use history, the two rewetted fens exhibit dissimilar methanogenic and, especially, methanotrophic community compositions. The methanotrophic community was generally under-represented among the prokaryotic communities and both fens show similarly low ratios of methanotrophic to methanogenic abundances. Since few studies have characterized methane-cycling microorganisms in rewetted fens, this study provides first evidence that the rapid and well re-established methanogenic community in combination with the low and incomplete re-establishment of the methanotrophic community after rewetting contributes to elevated sustained methane fluxes following rewetting.

Finally, this thesis demonstrates that dispersal limitation only slightly regulates the biogeographic distribution patterns of methanogenic microorganisms in natural environments and restored wetlands. Instead, their existence, adaption and establishment are more associated with the selective pressures under different environmental conditions. Salinity, pH and temperature are identified as the most important factors in shaping microbial community structure at different spatial scales (global versus terrestrial environments). Predicted changes in climate, such as increasing temperature, changes in precipitation patterns and increasing frequency of flooding events, are likely to induce a series of environmental alterations, which will either directly or indirectly affect the driving environmental forces of methanogenic communities, leading to changes in their community composition and thus potentially also in methane emission patterns in the future.

Zusammenfassung

Methan ist ein wichtiges Treibhausgas, das zum globalen Klimawandel beiträgt. Bedeutend für das globale Methanbudget sind unter anderem natürliche und wiedervernäßte Moore. Methanogene Archaeen (Methanogene) und Methan-oxidierende Bakterien (Methanotrophe) sind die biogenen Produzenten und Konsumenten von Methan. Daher nehmen sie global, und speziell in Mooren, eine Schlüsselrolle für das Methanbudget ein. Eine Vielzahl von Studien hat die Verteilung, Vielfalt und Zusammensetzung dieser Mikroorganismen in einzelnen Lebensräumen untersucht. Es bestehen jedoch Unsicherheiten in der Vorhersage, wie sie auf den globalen Wandel und auf die damit verbundenen Umweltveränderungen reagieren werden. Diese Unsicherheiten basieren unter anderem auf bislang fehlenden biogeographischen Untersuchungen, die globale und lokale Skalen kombinieren, und auf einem unzureichenden Verständnis dazu, ob und welche Umweltfaktoren speziell methanogene Gemeinschaften beeinflussen. Zudem gibt es trotz der Bedeutung von Projekten zur Moorwiedervernässung für das regionale und globale Treibhausgasbudget nahezu keine Untersuchungen zur Zusammensetzung und Verbreitung von methanogenen und methanotrophen Gemeinschaften in degradierten wiedervernäßten, eutrophen Niedermooren.

Das Ziel dieser Doktorarbeit ist es, unser Verständnis zur Reaktion der am Methanbudget beteiligten mikrobiellen Gemeinschaften auf den globalen Wandel und auf die damit einhergehenden Umweltänderungen zu verbessern. Die Arbeit untersucht daher zum einen die biogeographischen Muster methanogener Gemeinschaften und die ihnen zugrunde liegenden Umweltfaktoren auf verschiedenen räumlichen Skalen. Auf globaler Ebene wurde eine Meta-Analyse durchgeführt, die auf 94 global verteilten, öffentlichen Sequenzdatensätzen sowie den dazugehörigen Umweltdaten aus verschiedenen natürlichen Ökosystemen basiert. Hierzu gehören Böden, Seesedimente, Ästuare, marine Sedimente, hydrothermale Sedimente und Schlammvulkane. In Kombination mit einer globalen biogeographischen Karte zur Verbreitung methanogener Archaeen konnte diese Arbeit zeigen, dass biogeographische Muster von Methanogenen existieren.

Terrestrische Ökosysteme zeigen zudem eine höhere Diversität als marine Ökosysteme. Ästuare, Übergangszonen zwischen marinen und terrestrischen/ limnischen Ökosystemen, weisen die größte methanogene Diversität bei jedoch vergleichsweise geringen Methanemissionen auf. *Methanoculleus* und *Methanosaeta* (*Methanothrix*) sind die am häufigsten nachgewiesenen Taxa in marinen Lebensräumen, während *Methanoregula* in terrestrischen Ökosystemen dominiert. Auf lokaler Ebene wurden in dieser Arbeit zwei wiedervernässte, eutrophe Niedermoore im Nordosten Deutschlands verglichen, das von der Ostsee beeinflusste „Hütelmoor" und das Durchströmungsmoor „Polder Zarnekow". Beide Moore sind durch hohe Methanemissionen infolge der Wiedervernässung charakterisiert. Einhergehend mit unterschiedlichen geochemischen Bedingungen und unterschiedlicher Nutzungshistorie weisen diese beiden wiedervernässten Standorte in ihrer Zusammensetzung unterschiedliche methanogene und methanotrophe Gemeinschaften auf lokaler Ebene auf. Zudem ist die Gruppe der Methanotrophen innerhalb der prokaryotischen Gemeinschaften jeweils unterrepräsentiert und beide Moore zeigen ein vergleichbar niedriges Verhältnis von Methanotrophen im Vergleich zu Methanogenen. Diese Arbeit liefert erste Hinweise darauf, dass die schnelle und erfolgreiche Wiederbesiedlung durch Methanogene in Kombination mit einer offenbar schlecht etablierten methanotrophen Gemeinschaft zu den erhöhten Methanflüssen in beiden Mooren nach Wiedervernässung beiträgt.

Abschließend zeigt diese Arbeit, dass eine eingeschränkte Migration („dispersal limitation") die biogeographischen Verteilungsmuster von Methanogenen in natürlichen Ökosystemen kaum beeinflusst. Stattdessen werden Vorkommen und Anpassung von methanogenen Gemeinschaften vor allem durch den selektiven Druck verschiedener Umweltbedingungen reguliert. Die Umweltparameter Salzgehalt, pH-Wert und Temperatur wurden dabei als wichtigste Faktoren identifiziert, die die Verbreitung methanogener Gemeinschaften global bzw. speziell in terrestrischen Standorten beeinflussen. Es ist daher wahrscheinlich, dass prognostizierte Klimaveränderungen wie steigende Temperatur, Änderungen der Niederschlagsmuster und zunehmende Häufigkeit von Überschwemmungsereignissen zu Änderungen in der Zusammensetzung methanogener Gemeinschaften führen, die möglicherweise auch die Methanemissionsmuster beeinflussen werden.

Contents

1 Introduction

1.1 Motivation

Methane (CH_4), as a very important greenhouse gas (GHG), is 34 times stronger as a heat-trapping gas than CO_2 over a 100-year time scale according to the IPCC's Fifth Assessment Report (Pachauri et al 2014) and plays an increasing role in the on-going climate change (Saunois et al 2016). The terrestrial environments, especially wetlands, contributed nearly half of the total global methane emissions (Dean et al 2018). Climate change will potentially enhance methane emissions from these systems, and the increasing methane emissions could in turn boost further climate change, resulting in a positive climate feedback. Methane-cycling microorganisms, which include methanogenic archaea and methane oxidizing bacteria, are responsible for methane production and consumption in natural environments and restored wetlands. So far, considerable uncertainties still remain in predicting whether and how climate and environmental changes will affect these specific groups of microorganisms and thus the production and consumption of methane.

One of the essential aspects in answering this question is to integrate the composition, abundances, biogeographic patterns and distribution drivers of methane-cycling microbial communities in various habitats over different spatial scales. This knowledge would provide useful parameters in predicting the microbial contributions to total methane emissions from natural environments and restored wetlands. This thesis combined meta-analysis and site-specific molecular and biogeochemical analyses to get a better understanding of global and local distribution patterns of methane-cycling microorganisms, to evaluate the factors which control the distribution patterns, and to potentially supply predicting models with information of microbial responses to future climate and environmental changes.

1.2 Methane emissions in natural environments and restored wetlands

1.2.1 Methane emissions from natural environments

Methane is emitted from a variety of different natural and anthropogenic sources (Figure 1.1). Anthropogenic sources include rice agriculture, livestock, landfills, waste treatment, biomass burning and fossil fuel extraction and consumption. Natural methane sources comprise wetlands, freshwater systems, coastal sediments and oceans, methane hydrates, geological sources, wild animals and wildfires. The contribution of natural sources to global anthropogenic methane emissions since 1980 is estimated to range from 33 to 54% (Kirschke et al 2013).

Wetlands are the largest natural sources of methane, contributing nearly 30% of global atmospheric methane annually (Dean et al 2018). Methane emissions from wetlands mostly occur during warm seasons, and their relative contributions to the total release of methane vary largely due to the differences in vegetation and soil microbiota which will interfere with the methane production and consumption. Freshwater systems are also important components of global methane emission, with an estimated contribution of 6–16% to total natural methane emissions (Bastviken et al 2004). Within freshwater ecosystem, lakes emit greater methane than reservoirs and rivers. Although ocean covers around two-thirds of the earth's surface, ocean contributes a disproportionally small amount of methane to the global atmospheric budget, with about 8% to the natural sources of methane (Nazaries et al 2013). The majority (ca. 75%) of oceanic methane is produced in deeper sediment layers of productive coastal areas (Grunwald et al 2009). In estuaries, methane emissions to the atmosphere account for about 7.4% of the total oceanic emission (Middelburg et al 2002).

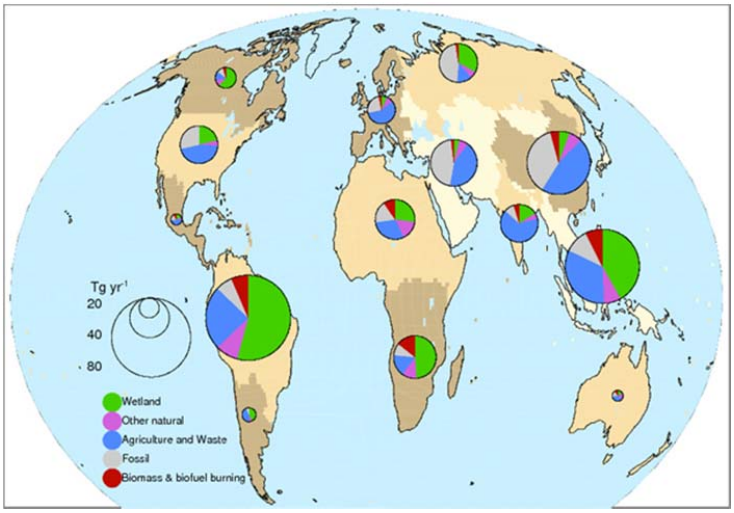


Figure 1.1 Global annual methane emissions (in Tg yr^{-1} for 2003–2012) for five emission categories (Saunois et al 2016).

In terms of formation mechanisms and/or conditions, the global methane budget is dominated by biogenic sources such as natural or disturbed wetlands, rice fields, forests, and oceans, intestines of ruminants and termites, and landfills. However, high uncertainty in biogenic emissions exist (Melton et al 2013, Schaefer et al 2016), especially regarding how natural methane production and consumption processes will be affected by the related environmental variables and their response to current and future climate change.

1.2.2 Methane emissions from restored peatlands

Peatlands are unique ecosystems. They contain a surface layer of at least 30–40 cm peat and are typically distributed in the northern latitudes and tropics. They represent approximately half (ca. 400 million ha) of the total global wetland area, covering 3% of the global land surface (Melton et al 2013, Mitsch et al 2009) (Figure 1.2). Peatland ecosystems contain up to 25% of the total global soil carbon, which is disproportional to their 3% coverage of the earth surface (Roulet et al 2007). As such,

peatlands are one of the key elements in the response and feedback to global climate change. Pristine or undisturbed peatlands act as long-term carbon sink with low CO_2 emission rates since the late Holocene (Roulet et al 2007). Currently, natural peatlands are moderate methane source as they are usually water-saturated, and the anaerobic conditions favor methane production (Vasander and Kettunen 2006). Although research data suggested that peatlands had only a minor impact on the overall carbon budget in the 20th century, recent estimates infer that the greenhouse gas released from peatlands will substantially increase in the future due to the direct and indirect impacts of climate change, permafrost thaw, wildfires, and anthropogenic activities (Frolking et al 2011).

Peatlands in natural and near-natural condition can actively form peat, thereby fixing CO_2 from the atmosphere and storing carbon in the soil, while degraded peatlands emit more CO_2 than they take up and become a net source of greenhouse gases. Anthropogenic perturbation to peatlands has extensively occurred worldwide, mainly by drainage for forestry and agriculture use, or mined for fuel and horticulture (Joosten and Clarke 2002). For example, more than 85% of the peatlands in Germany have been used or disturbed for agricultural activity (Silvius et al 2008). Such land-use changes have altered the peatland hydrology, resulted in peat oxidation and rapidly broke the natural greenhouse gas balance of the peatland. Peatland drainage led to an accelerated carbon loss in form of CO_2 emission, and consequently converts these peatlands to sources of CO_2 (Joosten 2009, Waddington et al 2002). Meanwhile, drainage shifts the methane production and consumption patterns and converts the ecosystem from methane sources to sinks (Minkkinen et al 2002). Water-level drawdown leads to decreased methane emissions by directly reducing production and enhancing oxidation rates (Kettunen et al 1999, Nykänen et al 1998). In addition, vegetation changes due to drainage and water table decline can also lower the methane emissions (Minkkinen and Laine 2006).

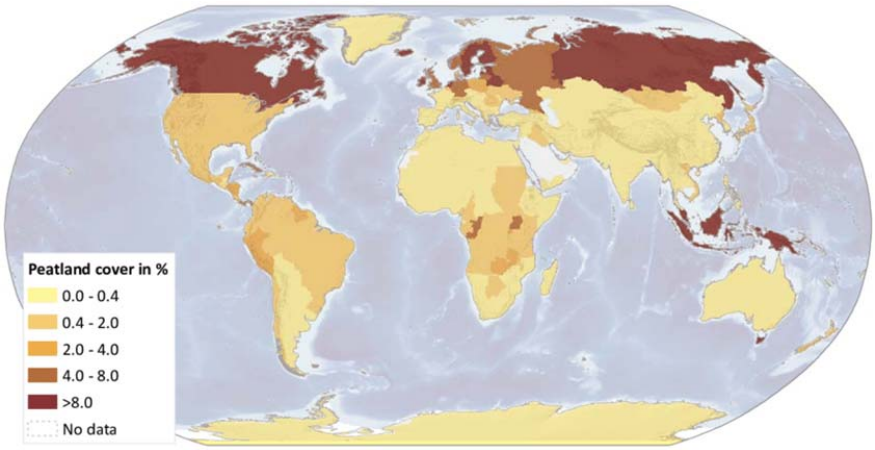


Figure 1.2 Global peatland distribution (Pittock et al 2015).

In recent years, peatland restoration is increasingly implemented to degraded sites. Peatland restoration aims to re-establish the peatland ecosystem to conditions prior to the disturbance, to reduce greenhouse gas emissions and to recover the C sink and ecological functions of pristine peatlands. The restoration methods can be divided into three main categories: water management (rewetting), re-vegetation and vegetation management. Rewetting is a commonly used restoration technique by raising the water table on drained peatlands to re-establish water saturated conditions, e.g. through blocking drainage ditches or simply flooding. Rewetting could potentially also lead to vegetation reconstruction. Meanwhile, peatland rewetting is a cost-effective carbon reducing approach compared to other available methods. Rewetting of peatlands often leads to reduced CO_2 but increased methane emissions, even several years after rewetting (e.g. Vanselow-Algan et al 2015, Waddington and Day 2007, Wilson et al 2009). Although high methane emissions are assumed to be a transient phenomenon for a limited duration (Cooper et al 2014, Joosten et al 2012), there is evidence showing considerable variation in the length of the duration and the factors that cause the magnitude of methane fluxes (Joabsson et al 1999).

1.3 Methane-cycling microorganisms

1.3.1 Taxonomy of methane-cycling microorganisms

Methanogenic archaea (methanogens) and methane-oxidizing bacteria (MOB, also known as methanotrophs) are the key players involved in methane-cycling due to their ability to either produce (by methanogens) or remove (by MOB) the greenhouse gas methane. Biogenic methane on earth is generated by a process called methanogenesis, which is the final step in the anaerobic degradation of organic matter. Methanogenesis typically takes place under anaerobic conditions and is performed by a group of strictly anaerobic archaea, namely methanogenic archaea, which convert CO_2 with H_2, methyl compounds (methanol, methylamines, methylsulfides), or acetate into methane (Figure 1.3) (Thauer et al 2008). Depending on the types of substrates, methanogens are typically classified as hydrogenotrophs, methylotrophs, and acetotrophs, respectively. Phylogenetically, methanogens belong to the phylum Euryarchaeota, which currently constitutes several families within seven known orders: Methanosarcinales, Methanomicrobiales, Methanopyrales, Methanocellales, Methanococcales, Methanobacteriales, and Methanomassiliicoccales (Liu and Whitman 2008, Oren and Garrity 2015, Paul et al 2012). Hydrogenotrophic methanogens are mainly from the orders Methanomicrobiales, Methanopyrales, Methanocellales, Methanococcales, and Methanobacteriales. The order Methanosarcinales has a broad substrate spectrum and is able to utilize all substrates except for formate (Liu 2010), while the order Methanomassiliicoccales consists exclusively of obligatory H_2-dependent methylotrophs (Lang et al 2015). Methanomassiliicoccales includes two broad phylogenetic clades which prevail in animal gastro-intestinal tracts and wetlands, respectively (Söllinger et al 2015). As obligate acetoclastic methanogens, *Methanosaeta* was suggested to be renamed as '*Methanothrix*' (Garrity et al 2011). Since "*Methanosaeta*" is more commonly used in the literature, *Methanosaeta* instead of *Methanothrix* will be used in this thesis. Recent studies have proposed some potential methanogenic clades like Verstraetearchaeota

and Bathyarchaeota that are outside the Euryarchaeota phylum (Evans et al 2015, Vanwonterghem et al 2016). Proof of methanogenic activity among these phyla is, however, lacking to date.

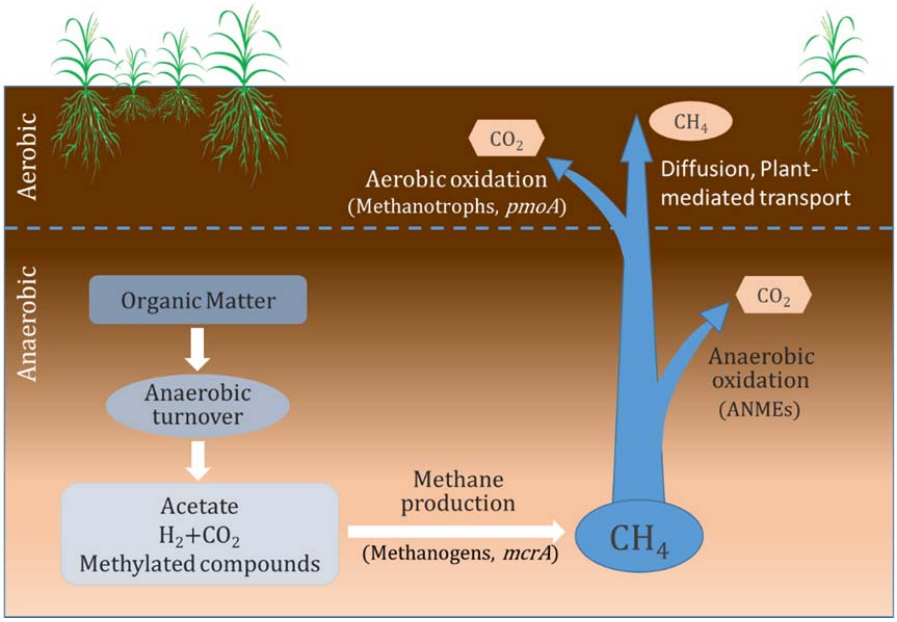


Figure 1.3 Conceptual illustration of microbial driven methane production and consumption. ANME: anaerobic methanotrophic archaea.

Methanotrophs provide a key function in the global carbon cycle by attenuating methane emissions to the atmosphere or even acting as a sink for atmospheric methane (Figure 1.3) (Lüke and Frenzel 2011, Semrau et al 2010, Tveit et al 2019). Aerobic methanotrophs are affiliated within the bacterial phyla of Proteobacteria and Verrucomicrobia. The verrucomicrobial methanotrophs seem to be restricted to extreme environments (Op den Camp et al 2009) and are represented by the family Methylacidiphilaceae. The proteobacterial methanotrophs are phylogenetically divergent and can be divided into type Ia and Ib (both Gammaproteobacteria) and type II (Alphaproteobacteria) (Semrau et al 2010). Type I aerobic methanotrophs belong to the family of Methylococcaceae with the genera *Methylococcus*,

Methylocaldum, *Methylomicrobium*, *Methylosphaera*, *Methylomonas*, *Methylobacter*, *Methylosarcina*, *Methylothermus*, and *Methylohalobius*. Type II is affiliated to the family Methylocystaceae, with the genera *Methylocystis*, *Methylosinus*, *Methylocella*, and *Methylocapsa*. Type I and type II methanotrophs differ not only in phylogenetic classification but also in several biochemical characteristics, such as the pathway of carbon assimilation (ribulose monophosphate pathway in type I and serine pathway in type II) and the dominant phospholipid fatty acids (unsaturated PLFAs with 16 and 14 carbon atoms in type I and with 18 carbon atoms in type II) (Conrad 2007). All aerobic methanotrophs activate methane with a methane monooxygenase (MMO), which requires molecular O_2 and reducing equivalents (reduced cytochrome C or NADH), and results in the production of methanol (Lieberman and Rosenzweig 2004, Murrell et al 2000). The MMO occurs as a particulate, membrane-bound form (pMMO) or in a soluble, cytoplasmic form (sMMO). The pMMO is universal to all aerobic methanotrophs, except for *Methylocella* spp. which only have a sMMO (Dedysh et al 2000).

In addition, methane emission can also be attenuated by a group of anaerobic methanotrophic archaea (ANME) which use sulfate, nitrate or metals as final electron acceptors for anaerobic oxidation of methane (AOM) (Figure 1.3) (Knittel and Boetius 2009). This group received increasing research efforts in recent years. The commonly identified AOM constitutes of sulfate-dependent clades like ANME-1, ANME-2a/2b, ANME-2c, and ANME-3 clades, nitrate-dependent ANME-2d and ferric iron-related Methanoperedenaceae (Evans et al 2019, Haroon et al 2013). Anaerobic methane oxidation is a significant process especially in marine environments, but also in various freshwater sediments (Knittel et al 2019).

1.3.2 Biogeography and environmental factors influencing methane-cycling microorganisms

Biogeography is the study of the distribution of organisms across space and time, which aims to determine where organisms live, at what diversity (composition and

abundance), and why they occur (Lomolino et al 2017). A growing body of research supports the idea that microorganisms exhibit biogeographic patterns (e.g. Hanson et al 2012, Lüke et al 2010, Lutz et al 2016, Martiny et al 2006, Nelson et al 2016). Although the mechanisms shaping the microbial biogeographic patterns are not well known (Meyer et al 2018), environmental selection and dispersal limitation are commonly accepted as important regulating factors (Hanson et al 2012). Environmental selection results in microorganisms that are relatively better adapted to the local conditions, as long as microorganisms can vary in their response to those conditions, while dispersal limitation refers to the question whether microorganisms show restriction in movement from one location to another and successful establishment. The relative importance of these two factors may vary with location and spatial scales (Hanson et al 2012).

Methane-cycling microorganisms have been detected in various natural and disturbed environments. Methanogens are abundant in a wide variety of anaerobic habitats such as wetlands, marine sediments, lake sediments, flooded soils, and rice paddies (e.g., Conrad et al 2014, Großkopf et al 1998, Merila et al 2006, Newberry et al 2004), as well as in some extreme habitats such as hydrothermal vents and permafrost soils (e.g., Reed et al 2009, Wagner and Liebner 2009), and likely show environmental preferences. For example, sulfate-rich sediments such as marine sediments allow methylotrophic methanogens to produce limited amount of methane as they utilize methylated compounds which are not utilized efficiently by the sulfate-reducing bacteria, while freshwater sediments with lower sulfate concentrations are usually co-dominated by acetoclastic and hydrogenotrophic methanogens (Liu and Whitman 2008). In some natural habitats, methanogens are also present in micro-oxic environments (Wagner 2017). For example, methanogens from the order Methanocellales were found predominantly in the transiently oxic rice rhizosphere (Erkel et al 2006).

Similarly, methanotrophs appear to be ubiquitous in various environments. Nearly all samples taken from muds, swamps, rivers, rice paddies, oceans, ponds, meadows,

deciduous woods, streams, sewage sludge, and several other environments contained methanotrophs (Hanson and Hanson 1996). Type II methanotrophs generally prefer environments characterized by high methane, low oxygen, and limited combined nitrogen and copper concentrations, while type I methanotrophs are dominant in environments where methane is limited and the level of nitrogen and copper are relatively high (Hanson and Hanson 1996). Furthermore, representatives of type I methanotrophs were found particularly predominant in freshwaters and sediments (Costello et al 2002, Rahalkar and Schink 2007). A recent study showed specific type II methanotrophs in soil which oxidize atmospheric methane (Tveit et al 2019). Most methanotrophs are mesophilic and neutrophilic, however, there are also some methanotrophic bacteria living in extreme environments like hot spring, Antarctic lake, and volcanic mud (e.g., Bowman et al 1997, Dunfield et al 2007, Islam et al 2008, Tsubota et al 2005).

In pristine peatlands, molecular surveys have identified a diverse, but mainly uncultured, methanogenic community. Acidic bogs were found to contain uneven and poorly diverse methanogenic communities which are dominated by hydrogenotrophic methanogens (mainly Methanoregulaceae). In fens, the prevailing methanogens are members of Methanoregulaceae and acetoclastic Methanosaetaceae (Bridgham et al 2013). Vertical stratification of methanogenic communities in pristine peatlands was also detected. For instance, Galand and co-workers observed a vertical shift from hydrogenotrophic to acetoclastic methanogens with increasing depth in a Finnish fen (Galand et al 2002). In the interface between oxic and anoxic layers of peatlands, or in the soil horizon where the water table fluctuates, the simultaneous presence of methane and oxygen creates favorable conditions for methanotrophs. For instance, a *Sphagnum*-dominated peatland was inhabited by a high biodiversity of both type I and II methanotrophs (Kip et al 2011). However, anthropogenic or natural disturbances could lead to shift in the methane production and consumption patterns. With drainage, previously anoxic peat soils are exposed to oxygen, which reduces methane production by methanogenic archaea and may limit methane consumption by aerobic methanotrophs through substrate depletion (Jaatinen et al 2005, Yrjälä et

al 2011). For example, Urbanová et al found that a significant decrease in methane emission and potential methane production after drainage coincided with changes in the abundance and diversity of methanogens as compared to pristine sites, suggesting the linkage between ecological function and the methane-cycling community (Urbanová et al 2013). Rewetting usually modifies the methanogenic and methanotrophic communities as their ecological niche is dependent on the position of the water table (Francez et al 2000). Water table changes may also cause changes in vegetation which could further regulate the composition of the methane-cycling communities. A study of forestry-drained peatlands, for example, indicated that the abundances of methanogens and methanotrophs in restored peatlands were different with those in natural sites and that these variations were correlated with methane emission (Juottonen et al 2012).

Within single habitats or limited spatial scales, the distribution of methanogenic and methanotrophic communities was ascribed to some environmental parameters. For methanogens, salinity was found to be a factor which regulates methanogenic activity. Pattnaik et al. (2000) demonstrated lower methane production in saline soils as compared to nonsaline soils, which was attributed to the lower soil methanogenic population and their activity in saline conditions. pH is another important factor controlling methanogens as low pH generally hampers acetotrophic methanogenesis by reducing acetate dissociation (Kotsyurbenko et al 2007, Megonigal et al 2004). Temperature can influence the structure and activity of methanogenic community as well (Tveit et al 2015). A study in peatlands identified that, under climate warming, methanogen abundance decreased and this decrease resulted in a reduction on potential methane production (Peltoniemi et al 2016). Water table was also found to be an environmental control for methanogenic activity. Yrjälä et al. (2011) showed long-term water table lowering in a boreal fen was associated with decreased methane emissions and methane production potential. Along the water table lowering gradient, the diversity and activity of the methanogenic community decreased as well. The methanotrophic community was frequently found to be driven by methane concentration, oxygen availability, temperature and salinity. Methanotrophs can be

divided into two groups according to affinity for methane. High-affinity methanotrophs are found mainly in aerobic upland soils (Maxfield et al 2008), while low-affinity methanotrophs are more widespread. A study on sediments from Lake Washington showed that different methanotrophic species persisted under different oxygen tensions, indicating oxygen availability is one of major factor determining specific partnerships in methane oxidation (Hernandez et al 2015). Lofton et al found that methane oxidation significantly increased with elevated temperature (Lofton et al 2014). In addition, a review of methane emissions from estuaries revealed that significant methane oxidation by methanotrophs only occurs at low salinities (Abril and Borges 2005, Osudar et al 2018).

Some recent studies addressed the influence of dispersal limitations on methane-cycling microbial communities (Auguet et al 2010, Barreto et al 2014, Lüke et al 2010, Yavitt et al 2011). Yavitt et al. (2011) found that in six peatlands in North America, turnover in methanogenic community composition between sites was more strongly driven by the variation in soil pH and annual temperature than by geographic distance. Coincidently, another study in rice paddies also suggested that the methanotrophic communities were mainly shaped by environmental conditions rather than geographic location (Lüke et al 2010). However, these results are derived from studies in local spatial scales which require to be evaluated at larger scale. A better understanding of methane-cycling microbial communities in a changing world requires a systematic and integrated exploration of the biogeographic patterns of methane-cycling microbial communities, the correlations between the community structure and the geographical locations or environmental conditions, at both global and local scales from various habitats. In addition, even though rewetted wetlands can act as hotspots of methane in a long term, the distribution and abundance as well as their drivers of methane-cycling microorganisms in rewetted peatlands is rarely described.

1.4 Molecular and ecological tools for studying methanogens and methanotrophs

Consistent with most environmental microorganisms, the majority of the methane-cycling microorganisms cannot or was not cultivated under laboratory conditions. Culture-independent molecular methods, such as DNA or RNA based fingerprints, have been extensively used and greatly expanded our knowledge on the diversity of the environmental methane-cycling community. In recent years, the high-throughput sequencing (NGS) technologies on amplicons enabled researchers to simultaneously sequence hundreds to thousands of samples (Caporaso et al 2011). The massive data from NGS technology also allowed to capture detailed information at higher resolution to systematically investigate the dynamics of methane-cycling microbial community, their ecological rules, and whether microbial ecology plays a significant role in mediating or driving biogeochemical function (Mackelprang et al 2016). The quantitative polymerase chain reaction (qPCR) is another DNA-based technique to determine the copy number or, in other words, abundance of methane-cycling microorganisms from environmental samples.

Similar to most prokaryotes, methanogenic and methanotrophic communities are widely described through targeting the 16S rRNA gene as a phylogenetic marker gene (Tringe and Hugenholtz 2008). In addition, the two groups are often probed via functional gene markers, and sequence analysis of protein-encoding genes has particular advantage for detecting genetic variations (Lüke and Frenzel 2011). For methanogenesis, the CH_4-producing reaction is catalyzed by the methyl-CoM (methyl-coenzyme M) reductase, which converts methyl-CoM and HS-HTP (N-7-mercaptoheptanoyl-O-phospho-L-threonine) to methane. This reaction is universal to all methanogens, independently of the primary substrate (Conrad 2007). As such, this key enzyme is an ideal target for specifically detecting methanogens. The *mcrA* gene, coding for the alpha subunit of the methyl-CoM reductase, is able to provide a congruent phylogeny to that based on the 16S rRNA gene (Lueders et al 2001).

Similarly, the *pmoA* gene, encoding a subunit of the particulate methane monooxygenase (pMMO), is highly conserved and was shown to be an excellent phylogenetic marker for methanotrophs in various environments (Dumont and Murrell 2005, Lüke and Frenzel 2011). In recent years, massive *pmoA* gene sequences have been generated to explore methanotrophic communities from various habitats worldwide. These newly generated *pmoA* gene sequences, especially new methanotrophic isolates and sequences from uncultured MOB, should be incorporated as new references to probe the genetic novelty of methanotrophs in complex environments. To reasonably analyze methanotrophic communities at nucleotide level, for example, research effort is required to cover numerous newly generated nucleotide *pmoA* sequences, and to evaluate and update a precise *pmoA* gene cutoff value for family, genus and species levels. Therefore, *pmoA* reference databases and relevant cutoffs need to be updated.

1.5 Objectives

The main objective of this thesis is to extend our ability in predicting the response of methane-cycling microbial communities in natural environments and restored wetlands to climate and environmental changes. The thesis is based on the combination of molecular and biogeochemical analysis, exploring the composition, abundances, and biogeographic patterns of methane-cycling communities and their drivers in the environment on various spatial scales from local to global. The following scientific questions have been addressed:

- Do methane-cycling microorganisms show biogeographic patterns? If so, what are their global and local biogeographic patterns and diversities in natural environments and restored wetlands?

- Is dispersal limitation or habitat filtering shaping the distribution patterns of methane-cycling communities?

- What are important environmental factors in regulating methane-cycling microbial community composition at global and local scales?

An additional aim of the thesis was to evaluate and update the cutoff values for processing methanotrophic *pmoA* gene sequences at the nucleotide level taking into account the substantial number of recently isolated aerobic methanotrophs including the phylum Verrucomicrobia.

1.6 Study sites

1.6.1 Globally distributed natural environments

To get a better understanding of the biogeographic distribution patterns as well as drivers of methanogenic communities worldwide, we collected data from 94 sites in multiple natural environments (Figure 2.1). The 94 sites were globally distributed, however the vast areas of the Russian and Canadian Subarctic and Arctic as well as Australia were poorly represented. The 94 sites were grouped into six habitats: soil (28 sites), lake sediment (15 sites), estuary (14 sites), marine sediment (22 sites), hydrothermal sediment (9 sites), and mud volcano (6 sites). The *mcrA* sequences, geographical coordinates and environmental parameters (pH, salinity, and elevation, mean annual air temperature (MAAT) and mean annual precipitation (MAP)) of these sites were retrieved and extracted accordingly from sequence databases and papers. Details can be found in supplementary Table A.1.

1.6.2 Rewetted peatlands

Methane-cycling communities and their environmental drivers at local scale were addressed at the example of restored wetlands in northeastern Germany which are important ecosystems for the regional and global methane budget. We examined the

methanogenic and methanotrophic community composition and abundance in relation to post-flooding geochemical conditions in two rewetted fens (Figure 3.1).

The research site Hütelmoor, which is part of the nature reserve 'Heiligensee and Hütelmoor' in northeastern Germany, is a coastal, mainly minerotrophic, fen complex (Figures 3.1a and b). The fen is separated from the Baltic Sea by a narrow dune dike. In the 1970s, the fen had been drained with water levels down to 1.60m below surface which caused aerobic decomposition and concomitant degradation of the peat (Voigtländer et al 1996). In 2009, after the installation of a weir at the outflow of the catchment (Weisner and Schernewski 2013), the site has been fully flooded year-round with an average water level of 0.6m above the peat surface, and subsequently the annual average CH_4 flux increased 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) is a nutrient rich fen located in the valley of the river Peene in Mecklenburg-Vorpommern (northeastern Germany, Figures 3.1a and c). Drainage of the fen was initialized in the 18th century and strongly intensified in the mid-1970s with the water table down to more than 1m below surface (Hahn-Schöfl et al 2011). The rewetting process was initiated in 2004 by simply opening a dike. After rewetting, the water table increased to 0.1 – 0.5m above the peat surface and the methane flux rates increased to ~0.21 kg CH_4 m^{-2} a^{-1} (Augustin and Joosten 2007).

1.7 Thesis organization

This cumulative dissertation consists of an introductory part (Chapter 1), which provides the scientific background, the research approach, and the aims and objectives of the thesis. The obtained results are presented in three manuscripts (chapter 2 - 4) that have been published as original research articles in international peer-reviewed journals. Chapter 5 synthesizes the major results. Table 1.1 gives an overview of the respective publications.

Table 1.1: Overview of the publications presented within this thesis.

Manuscript	Publication	Manuscript status
I	**Wen, X.**, Yang, S., Horn, F., Winkel, M., Wagner, D., and Liebner, S.: *Global biogeographic analysis of methanogenic archaea identifies community-shaping environmental factors of natural environments*	Published in: *Frontiers in Microbiology* DOI: 10.3389/fmicb.2017.01339
II	**Wen, X.**, Unger, V., Jurasinski, G., Koebsch, F., Horn, F., Rehder, G., Sachs, T., Zak, D., Lischeid, G., Knorr, K., Böttcher, M., Winkel, M., Bodelier, P., and Liebner, S.: *Predominance of methanogens over methanotrophs in rewetted fens characterized by high methane emissions*	Published in: *Biogeosciences* DOI: 10.5194/bg-15-6519-2018
III	**Wen, X.**, Yang, S., and Liebner, S.: *Evaluation and update of cutoff values for methanotrophic pmoA gene sequences*	Published in: *Archives of Microbiology* DOI: 10.1007/s00203-016-1222-8

1.8 Summary of the included manuscripts and contribution of the co-authors

Manuscript I (published in *Frontiers in Microbiology*, 2017)
Global biogeographic analysis of methanogenic archaea identifies community-shaping environmental factors of natural environments (see chapter 2)

Authors:
Xi Wen[1, 2], Sizhong Yang[1, 3], Fabian Horn[1], Matthias Winkel[1], Dirk Wagner[1, 4], and Susanne Liebner[1, 4]

Summary:
In order to get a better understanding of global distribution patterns and environmental drivers of methanogenic communities in natural environments, such as soils, lake sediments, estuaries and marine sediments, we performed a global scale meta-analysis targeting the biogeographic patterns and environmental controls of methanogenic communities using 94 globally distributed public *mcrA* gene datasets. The results showed a global pattern of methanogenic archaea that is more associated with habitat filtering than with geographical dispersal. Salinity was identified as the control on methanogenic community composition at global scale whereas pH and temperature are the major controls in non-saline soils and lakes. The importance of salinity for structuring methanogenic community composition is also reflected in the biogeography of methanogenic lineages and the physiological properties of methanogenic isolates. Linking methanogenic alpha-diversity with reported values of methane emission identifies estuaries as the most diverse methanogenic habitats with, however, minor contribution to the global methane budget. With salinity, temperature and pH our study identifies environmental drivers of methanogenic community composition facing drastic changes in many natural environments at the moment.

Contribution of the coauthors:

Susanne Liebner and Xi Wen designed the study. Xi Wen and Sizhong Yang collected and analyzed the data. Xi Wen, Sizhong Yang, and Fabian Horn performed the statistical analysis. Xi Wen, Matthias Winkel, and Sizhong Yang did the phylogenetic correction. Xi Wen interpreted the results and wrote the paper with contributions of all co-authors.

Manuscript II (published in *Biogeosciences*, 2018)
Predominance of methanogens over methanotrophs in rewetted fens characterized by high methane emissions (see chapter 3)

Authors:
Xi Wen[1, 2], Viktoria Unger[5], Gerald Jurasinski[5], Franziska Koebsch[5], Fabian Horn[1], Gregor Rehder[6], Torsten Sachs[7], Dominik Zak[8, 9], Gunnar Lischeid[10, 11], Klaus-Holger Knorr[12], Michael E. Böttcher[13], Matthias Winkel[1, 14], Paul L. E. Bodelier[15], and Susanne Liebner[1, 4]

Summary:
To advance our understanding of the distribution and abundance of methane-cycling microorganisms in rewetted peatlands, which shows sustained elevated methane emission after rewetting, we compared the community composition and abundance of methane-cycling microorganisms in relation to peat porewater geochemistry in two rewetted fens in northeastern Germany. The results demonstrated that even though the two rewetted fens differ in geochemical conditions and microbial community composition, they display 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 methane emission rates, we found that pristine wetlands have a higher abundance of methanotrophs than measured in the fens in this study, suggesting that the rapid (re)establishment of methanogens and slow (re)establishment of methanotrophs contribute to prolonged increased methane emissions following rewetting.

Contribution of the coauthors:

Franziska Koebsch, Susanne Liebner, Gerald Jurasinski, Xi Wen, and Viktoria Unger formulated the research questions and study design. Franziska Koebsch, Matthias Winkel, Susanne Liebner, and Torsten Sachs performed field work. Susanne Liebner, Gerald Jurasinski, Xi Wen, Viktoria Unger, and Torsten Sachs visualized the data and prepared maps. Susanne Liebner, Michael E. Böttcher, Klaus-Holger Knorr, Gunnar Lischeid, and Dominik Zak provided analytical data measurements. Fabain Horn conducted the bioinformatics analyses. Paul L. E. Bodelier provided the incubation data and prepared the Supplement figure. All authors contributed to the discussion and interpretation of the data and the writing of the paper. Viktoria Unger and Xi Wen contributed equally to the writing of the paper and prepared most parts of the manuscript.

Manuscript III (published in *Archives of Microbiology*, 2016)
Evaluation and update of cutoff values for methanotrophic *pmoA* gene sequences (see chapter 4)

Authors:
Xi Wen[1, 2], Sizhong Yang[1, 3], and Susanne Liebner[1, 4]

Summary:
In this study, we aimed to evaluate common *pmoA* gene cutoff values at the nucleotide level and to establish such values for the genus and family level taking into account recently isolated methanotrophs. We compared the similarities between the *pmoA* gene and the corresponding 16S rRNA gene sequences of 77 described species covering gamma- and alpha-proteobacterial methanotrophs (type I and type II MOB, respectively) as well as methanotrophs from the phylum Verrucomicrobia. We updated and established the weighted mean *pmoA* gene cutoff values of 86, 82, and 71% on the nucleotide level corresponding to the 97, 95, and 90 % similarity of the 16S rRNA gene. Based on these cutoffs, the functional gene fragments can be entirely processed at the nucleotide level throughout software platforms such as Mothur or QIIME.

Contribution of the coauthors:

Susanne Liebner, Sizhong Yang and Xi Wen designed the study. Xi Wen and Sizhong Yang collected and analyzed the data. Xi Wen interpreted the results and wrote the paper with contributions of the two co-authors.

List of author's affiliations:

[1] Section 5.3 Geomicrobiology, GFZ German Research Centre for Geosciences, Helmholtz Centre Potsdam, Telegrafenberg, 14473 Potsdam, Germany

[2] College of Electrical Engineering, Northwest Minzu University, Lanzhou, 730070, China

[3] State Key Laboratory of Frozen Soil Engineering, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou, China

[4] University of Potsdam, Institute of Biochemistry and Biology, 14469 Potsdam, Germany

[5] Landscape Ecology and Site Evaluation, Faculty for Agricultural and Environmental Sciences, Rostock University, 18059 Rostock, Germany

[6] Department of Marine Chemistry, Leibniz Institute for Baltic Sea Research, 18119 Warnemünde, Germany

[7] Section 1.4 Remote Sensing, GFZ German Research Centre for Geosciences, Helmholtz Centre Potsdam, Telegrafenberg, 14473 Potsdam, Germany

[8] Department of Bioscience, Aarhus University, 8600 Silkeborg, Denmark

[9] Department of Chemical Analytics and Biogeochemistry, Leibniz Institute of Freshwater Ecology and Inland Fisheries, 12587 Berlin, Germany

[10] Institute of Landscape Hydrology, Leibniz Center for Agricultural Landscape Research, 15374 Münchberg, Germany

[11] Institute of Earth and Environmental Science, University of Potsdam, 14476 Potsdam, Germany

[12] Institute of Landscape Ecology, University of Münster, 48149 Münster, Germany

[13] Geochemistry and Stable Isotope Biogeochemistry, Leibniz Institute for Baltic Sea Research, 18119 Warnemünde, Germany

[14] Water and Environmental Research Center, Institute of Northern Engineering, University of Alaska Fairbanks, 306 Tanana Loop, Fairbanks, AK 99775, USA

[15] Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg 10, 6708PB Wageningen, the Netherlands

2 Global biogeographic analysis of methanogenic archaea identifies community-shaping environmental factors of natural environments

2.1 Abstract

Methanogenic archaea are important for the global greenhouse gas budget since they produce methane under anoxic conditions in numerous natural environments such as oceans, estuaries, soils and lakes. Whether and how environmental change will propagate into methanogenic assemblages of natural environments remains largely unknown owing to a poor understanding of global distribution patterns and environmental drivers of this specific group of microorganisms. In this study, we performed a meta-analysis targeting the biogeographic patterns and environmental controls of methanogenic communities using 94 public *mcrA* gene datasets. We show a global pattern of methanogenic archaea that is more associated with habitat filtering than with geographical dispersal. We identify salinity as the control on methanogenic community composition at global scale whereas pH and temperature are the major controls in nonsaline soils and lakes. The importance of salinity for structuring methanogenic community composition is also reflected in the biogeography of methanogenic lineages and the physiological properties of methanogenic isolates. Linking methanogenic alpha-diversity with reported values of methane emission identifies estuaries as the most diverse methanogenic habitats with however, minor contribution to the global methane budget. With salinity, temperature and pH our study identifies environmental drivers of methanogenic community composition facing drastic changes in many natural environments at the moment. However,

consequences of this for the production of methane remain elusive owing to a lack of studies that combine methane production rate with community analysis.

2.2 Introduction

Methane (CH_4) is a major greenhouse gas. Its emission from natural environments such as wetlands, oceans, and sediments accounts for over 70% of atmospheric methane globally (IPCC, 2007). An assessment of published data revealed different methane emission rates for wetlands, lakes, rivers, estuaries and oceans (in decreasing order, supplementary Figure A.1). Natural wetlands alone account for 62% of the biogenic CH_4 production (Kirschke et al 2013, Nazaries et al 2013) and wetland emissions dominate the inter-annual variability of methane sources (Bousquet et al 2006). In contrast, the vast area of marine ecosystems only contribute about 8% to the natural sources of CH_4 (Nazaries et al 2013). Within the global oceanic methane emission, less than 10% is contributed by estuaries (Bange et al., 1994).

Methanogenesis, the biological formation of methane, is performed by methanogenic archaea which produce methane primarily from H_2/CO_2, methyl groups or acetate at anoxic conditions (Thauer et al 2008). This reaction is catalyzed by the methyl-coenzyme M reductase (MCR). The *mcrA* gene encoding a subunit of this enzyme is a commonly used gene marker in molecular surveys (Bridgham et al 2013, Conrad 2007). The advantage of the *mcrA* gene marker is to capture both the phylogenetic and functional signatures of methanogens, offering a high sequencing depth for this particular function (Borrel et al 2013, Luton et al 2002, Yang et al 2014). A large number of *mcrA* sequences were retrieved from a variety of natural environments. The public *mcrA* data set allows for extracting general ecological patterns and investigating the shaping environmental gradients at global and regional scales. Additionally, a database summarizing the physiological properties of 152 methanogenic isolates is available (http://metanogen.biotech.uni.wroc.pl/) (Jabłoński et al 2015). Recently, genome binning revealed unusual *mcrA* sequences in the new class of Methanofastidiosa (Nobu et al 2016) and the new phyla of Bathyarchaeota

(Evans et al 2015) and Verstraetearchaeota (Vanwonterghem et al 2016). These new findings expanded our knowledge about the diversity of potential methanogens but did not obscure the applicability of the *mcrA* gene as a molecular marker for the large majority of methanogenic communities.

To date, methanogenic communities have been detected in wetlands, sediments, permafrost areas, rice paddies, digesters, geothermal springs and hydrothermal vents (Conrad 2007, Thauer et al 2008, Wagner and Liebner 2009). The methanogenic community structure was found to be associated with environmental pH, temperature, salinity, ground water level and vegetation dynamics at different spatial and temporal scales (Cui et al 2015, Frank-Fahle et al 2014, Liebner et al 2015, McCalley et al 2014, Megonigal et al 2004, Milferstedt et al 2010). For example, acetoclastic methanogenesis is generally hampered by low pH as it reduces the acetate dissociation (Kotsyurbenko et al 2007, Megonigal et al 2004). The vegetation can supply labile, high-quality organic carbon to fuel methanogens in the form of root exudates or detritus so that plant exudates generally favor acetoclastic methanogens primarily in fens (Bridgham et al 2013). Sulfate from seawater inhibits methane production in tidal wetlands, and salinity has consequently been used as a general predictor for methane emissions (Holm et al 2016). A study on Tibetan lake sediments showed that increasing salinity inhibits hydrogenotrophic methanogens but enhances acetoclastic methanogenesis (Liu et al 2016). These studies indicated environmental drivers for methanogenic communities, but have focused on single habitats or limited spatial scales.

Understanding the adaptation of methanogens to different environmental changes, however, requires a systematic and global exploration of the correlations between microbial community composition and environmental conditions (Lozupone and Knight 2007). At present, only a few studies address dispersal and habitat filtering of methanogenic communities (Auguet et al 2010, Barreto et al 2014). We hypothesize that methanogenic assemblages are mainly influenced by habitat filtering and that it is driven by global environmental controls. Considering that methane emission rates differ largely between natural ecosystems, the explicit integration of the composition,

diversity and biogeography of methanogenic assemblages in these ecosystems may be fundamental to determine the response of methane production to current and future climate change. This meta-study is performed to fill the gaps associated with methanogenic biogeography, diversity and its environmental controls by using publicly available *mcrA* sequence data and literature complemented through physiological data of methanogenic isolates.

2.3 Materials and methods

2.3.1 Data collection

We retrieved *mcrA* sequences available in GenBank (January 2016, http://www.ncbi.nlm.nih.gov/). For each hit, the original paper was checked and the according *mcrA* sequences were parsed by a custom Perl script. As we focused on natural environments, methanogenic *mcrA* sequences were obtained from natural habitats and classified as soil, lake-, estuary-, marine and hydrothermal sediments, and mud volcanos. Five libraries from next generation sequencing (NGS) were included in addition to sequences of clone libraries. Sequences were downloaded without taking into account relative abundance in the original dataset. Because sequences of clone libraries mainly covers the abundant phylotypes while NGS can capture much deeper diversity, we made a compromise in order to use the NGS data but mitigated a potential error due to different resolution of sequencing methods. Therefore, we only chose the representative sequences of abundant OTUs with a relative abundance higher than 1%. We further rejected those NGS sequences which failed the translation check from nucleotide to protein sequences or with a low quality (sequences < 250 bps). Finally, we constructed a dataset containing 4466 unique *mcrA* sequences from 94 globally distributed sites (Figure 2.1; Supplementary Table A.1 and A.2). In addition, we did not subtract the *mcrA* sequences of potential archaeal methanotrophs from the dataset, which was inevitably detected in the genomic survey (Conrad 2007). This part is beyond the focus of this study.

The geographical coordinates and environmental settings including pH, salinity, elevation, mean annual air temperature (MAAT) and mean annual precipitation (MAP) were extracted for each research sites considered in this study from the corresponding publications given that the data are available (see supplementary Table A.1). To account for missing environmental parameters across multiple studies, we qualitatively defined some environmental variables according to the site descriptions in the relevant literature, and then converted these category data into semi-metric numeric values, for example, we defined marine sediments, hydrothermal sediments, volcanic mud and soda lake sediment samples as "saline", soil and freshwater lake sediment samples as "nonsaline", and mangrove and estuary samples as "mixed" samples.

2.3.2 Raw sequence processing

The sequence processing was implemented with the Mothur software platform (Schloss et al 2009). Sequences from different libraries were pooled prior to processing. Sequences with a length less than 350 bp or more than 8 ambiguous bases were discarded. Subsequently, these sequences were aligned against a pre-aligned subset of *mcrA* sequences, which were retrieved from the FunGene database at http://fungene.cme.msu.edu/ (Fish et al 2013). Chimeric sequences were identified with the Mothur software using the uchime method (Edgar et al 2011) with the dataset itself as reference. Then, the valid *mcrA* gene nucleotide sequences were used to compute uncorrected pairwise distances between aligned DNA sequences and further assigned into operational taxonomic units (OTUs) at a cutoff of 84% which corresponds to 97% for 16S rRNA gene (Yang et al 2014). The abundance of each *mcrA* OTU was only accounted as presence and absence. We increased the accuracy of the taxonomic classification of the OTUs by considering both nucleotide and amino acid sequences. At DNA level, the taxonomic identity was assigned by the Mothur platform according to a

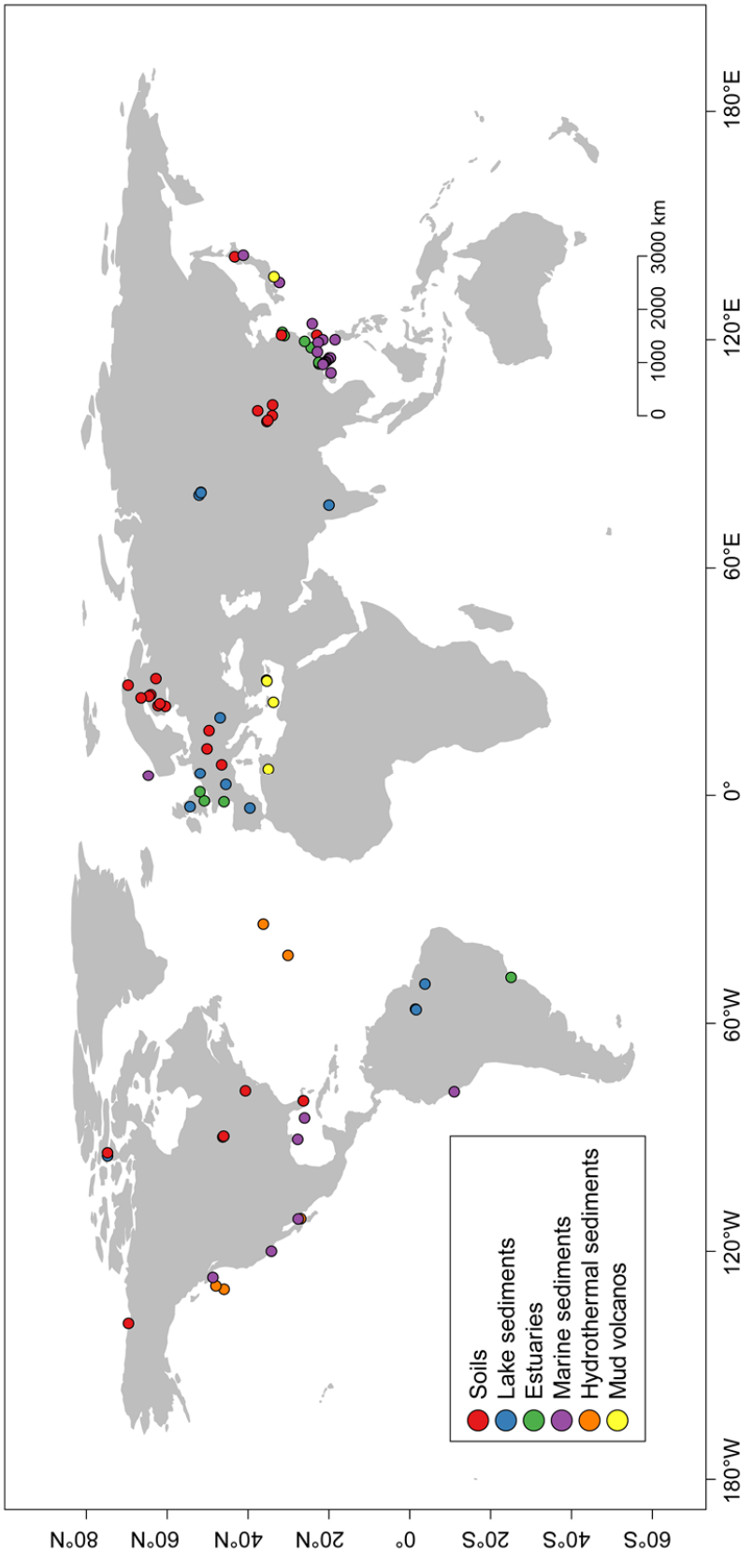


Figure 2.1 Location of the sites used for this study. The sites were grouped into 6 different categories according to their habitats and were labeled with different colors.

reference database (Yang et al 2014). At protein level, the aligned protein sequences were used to construct a tree in ARB, and then the taxonomic assignment was based on the corresponding database. If the assignment of an OTU was inconsistent, we manually blasted both the nucleotide and the protein sequence in NCBI and determined the final taxonomic identity by taking into account the query coverage (>95%), identity (>84%) and e-value (<1E-5). For protein sequences, the cutoff at the genus level referred to the threshold of 83.5% (Hunger et al 2011).

2.3.3 Ecological and statistical analysis

The statistical analysis was done by various *R* packages. Principal coordinates analysis (PCoA) ordinations were generated based on Jaccard distance matrices constructed using the vegan package v2.2.0 (Oksanen et al 2013). Permutational MANOVA (multivariate analysis of variance) was conducted to assess the source of variation in the Jaccard matrix based on (McArdle and Anderson 2001) in vegan with 10^4 permutations. The Jaccard distance measures are based on the presence/absence of the species, which is more suitable for our dataset as most studies only provided the representative sequences while the information about abundances is missing. The taxonomic incidence frequencies across habitats were visualized through bubble plots with the ggplot2 package (v1.0.0) (Wickham 2009). Hierarchical clustering analysis of the nonsaline soil and lake sediment communities was performed by the *R* function 'hclust' (R Core Team 2014). The obtained community clusters were described according to the pH and temperature regime of the original samples because using PCoA beforehand we identified both parameters pH and temperature to influence methanogenic community composition in nonsaline soils and lake sediments. The association of each methanogenic lineage with each of these clusters was determined using correlation-based indicator species analysis (Dufrêne and Legendre 1997). Indicator species are defined here as those that are both abundant in a specific type of habitat (specificity) and predominantly found in this type of habitat (fidelity). In this study, the indicator taxa, similar to the indicator species concept, for nonsaline soils

and lake sediments were picked according to an indicator value (IndVal value) by the *R* package labdsv (Roberts 2016) if the probability of obtaining an indicator value as high as observed over the specified iterations is less than 0.05. The Chao2 indices were calculated for each sample using the vegan package. The Wilcoxon rank sum test of Chao2 indices between habitats was performed by the *R* function 'wilcox.test' (R Core Team 2014). To consolidate the impact of habitat filtering on methanogenic archaea isolates, the physiological and biochemical characteristics of described methanogenic cultures were retrieved from the 'Methanogenic archaea database' (http://metanogen.biotech.uni.wroc.pl/) (Jabłoński et al 2015). Among them, the isolates with information of optimum NaCl requirement were filtered, categorized and plotted according to their isolation source.

To examine the influence of dispersal limitation on methanogenic community structure, a linear regression analysis was performed based on a geographical distance matrix and community Jaccard distance matrix by the *R* function 'lm' (R Core Team 2014). We performed Mantel and partial Mantel tests to evaluate the effects of dispersal limitation according to the two matrices again using the vegan package in R (Oksanen et al 2013). Further, multivariate spatial analysis (spatial PCA) was applied to 16 European soil and lake sediment samples based on Moran's I index to explore the spatial structure of methanogens by the function "multispati" in the *R* ade4 package (Dray and Dufour 2007). In addition, the Ward's Minimum variance clustering which was based on the Jaccard distance matrix was implemented on these 16 samples using the *R* function "hclust" (R Core Team 2014) and we further projected the clustering results on to a geographical map. The European shapefile for mapping at state level is available at the GSHHG Database (v2.3.6, https://www.ngdc.noaa.gov/mgg/shorelines/gshhs.html). The map was generated by using QGIS v2.18.2 (http://qgis.osgeo.org).

2.4 Results

2.4.1 Biogeography of methanogenic archaea in natural environments

The *mcrA* gene sequences from 94 globally distributed natural environments were retrieved. The location and ecosystem type of each of these 94 sites is depicted in Figure 2.1. The incidence (presence/absence) frequencies of methanogenic lineages were merged according to ecosystem type and illustrated in Figure 2.2. Briefly, *Methanoregula* is the most frequently observed taxon in soils, together with *Methanobacterium*, *Methanosaeta*, *Methanocella*, *Methanomassiliicoccus* and *Methanosarcina*. In estuary sediments, sequences from *Methanosaeta*, *Methanobacterium*, *Methanoregula* and *Methanoculleus* were commonly detected. Moreover in lake sediments, *Methanoregula* and *Methanosaeta* mainly occurred. In marine sediments, *Methanoculleus* and *Methanosaeta* are the most common lineages, followed by *Methanolinea*.

Even though many taxa were detected in different environments, some still show environmental preferences. *Methanoregula*, the taxon frequently occurring in non-marine and transitional environments (soils, lake sediments and estuaries), is absent from marine habitats (marine sediments, hydrothermal sediments and mud volcanos). *Methanobacterium* and *Methanocella*, which prevail in the non-marine and transitional environments, are rarely found in marine habitats. In contrast, *Methanococcoides*, as a predominant lineage in marine sediments, hydrothermal sediments and mud volcanos, is barely observed in soils and lake sediments. Moreover, *Methanogenium* and *Methanolacinia* are only observed in estuary and marine sediments whereas *Methanospirillum* and *Methanosphaerula* are only found in terrestrial environments. In addition, some specific taxa are exclusively found in hydrothermal sediments, including *Methanocaldocccus*, *Methanothermococcus*, *Methanopyrus*, *Methanotorris* and *Methermicoccus*. Although some lineages such as

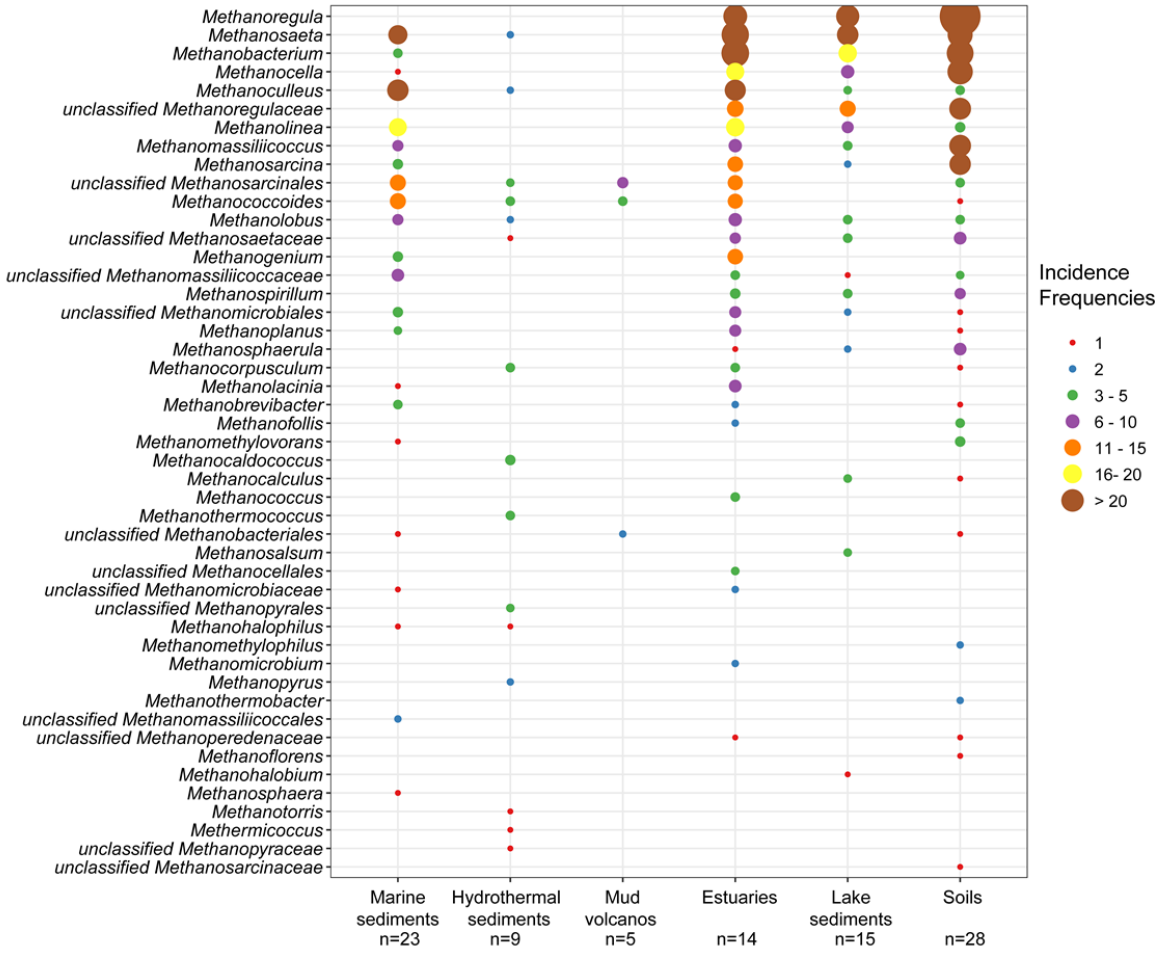


Figure 2.2 Bubble plot showing the incidence frequencies of methanogenic lineages in different natural environments. The rank order along the vertical axis corresponds to the decreasing total incidence frequencies of the lineages. The taxonomy is shown for the genus level. If an assignment to the genus level was not possible the next higher assignable taxonomical level was used. The number of samples (n) is given for each habitat.

Methanosaeta are present in most of the environments, no lineage can be regarded as omnipresent.

The highest richness of lineages occurred in estuary sediments which also harbor more even incidence frequencies of various lineages. In contrast, mud volcano and hydrothermal ecosystems display relatively low methanogenic diversity. Soils and lake sediments similar to estuaries are characterized by diverse methanogenic assemblages.

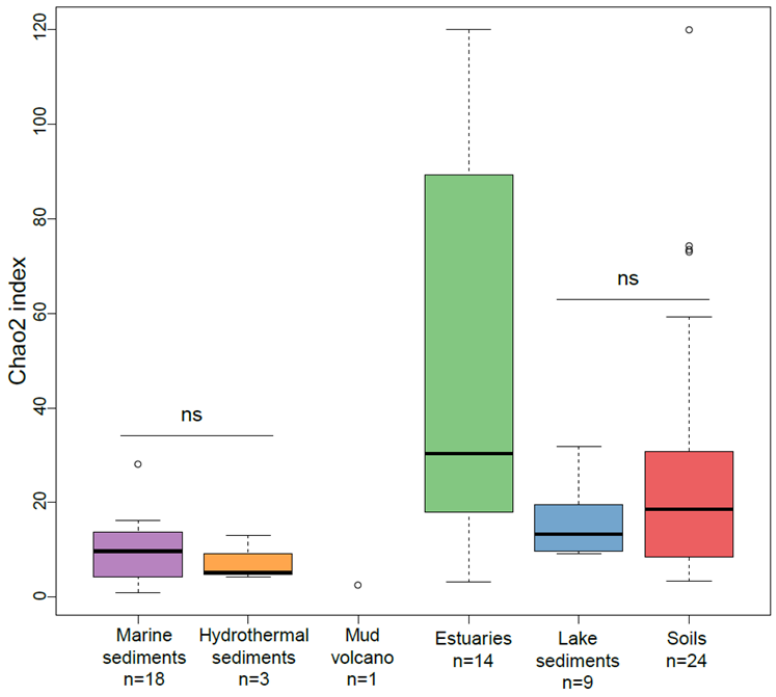


Figure 2.3 Box plot of Chao2 indices of the different ecosystem types. The plot is based on subsampled datasets containing 15 sequences for each site to make the comparison on alpha diversity measures more robust. The number of samples in each habitat is given as 'n' underneath the habitat label. The 'ns' donates no statistical significance in Wilcoxon test. The statistical result of alpha diversity at OTU level is given in Supplementary Table A.3.

2.4.2 Alpha-diversity of methanogenic archaea in natural environments

The richness of methanogenic archaea according to the Chao2 index varied largely between ecosystem types (Figure 2.3). For the purpose of directly comparing the alpha diversities and for obtaining a reasonable trade-off among samples, subsampling to 15 sequences for each site was performed. The Chao2 index shows that estuary sediments encompass the highest species richness of methanogens along the six ecosystem types (Supplementary Table A.3), which underlines the results of the bubble plot (Figure 2.2). Soils and lake sediments, showing lower richness than

estuary samples, have significantly higher Chao2 indices than marine sediments and hydrothermal sediments (Supplementary Table A.3) with no significant differences between marine sediments and hydrothermal sediments.

2.4.3 Global controls on methanogenic communities in natural environments

The 94 globally-distributed methanogenic communities were clustered into an ordination plot by applying PCoA based on Jaccard distance matrix. According to the PCoA analysis, the first and second axes together explain 16.3% of the total variance. The variations among the samples can thereby be largely explained by salinity (Figure 2.4). Since initial data on the salt concentrations were unavailable in some cases, we qualitatively assigned these samples as saline, mixed (intermediate) and nonsaline samples as described above. The saline and nonsaline samples effectively separate along the first axis. The mixed samples overall group in-between the saline and nonsaline samples. The permutational MANOVA based on the Jaccard distance matrix also suggests that salinity is the primary abiotic factor controlling the distribution of global methanogenic communities ($R^2 = 0.099$, $P < 0.001$) (Table 2.1).

Additionally, we checked for a potential relation between the isolation source of methanogenic pure cultures and the optimum concentration of NaCl for growth. The optimal concentration of NaCl of the methanogenic pure cultures demonstrated a decline from marine to estuaries, lake sediments and soil ecosystems (Supplementary Figure A.2). A few outlier isolates originate from soda lake sediments or hypersaline soils.

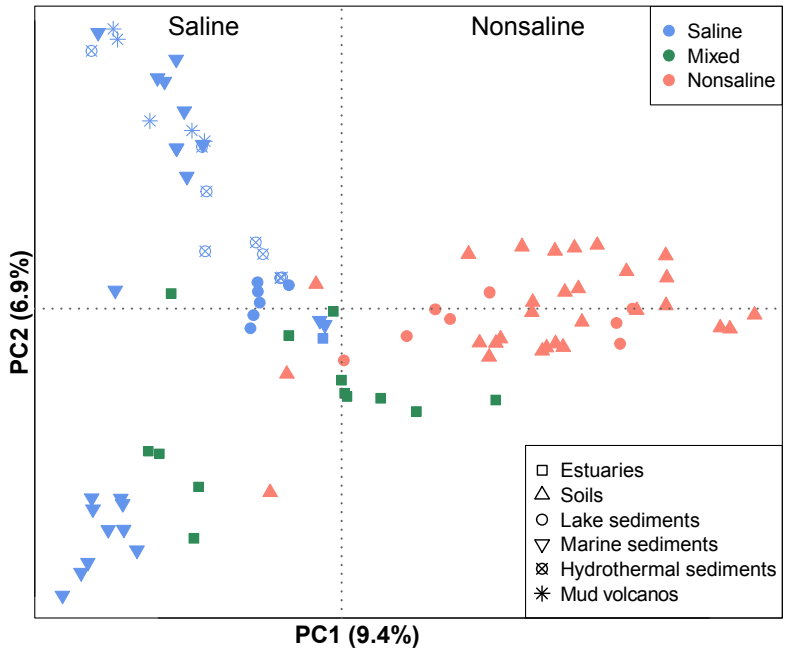


Figure 2.4 PCoA ordination based on the Jaccard distance matrix of methanogenic *mcrA* gene sequence libraries comparing 94 samples. The PCoA is colored by salinity: The red symbols indicate nonsaline environments, the blue ones indicate saline environments, and the green ones indicate intermediate environments. Different symbols depict different environments. The percentage of the variation explained by the plotted principal coordinates is indicated on the axes.

2.4.4 Environmental controls and methanogenic indicator taxa in nonsaline soils and lake sediments

On a global scale, methanogenic communities from nonsaline soils and lake sediments cluster closely (Figure 2.4) so that we further analyzed the environmental controls of methanogenic communities from these two habitats which account for 33 study sites in total. Community based cluster analysis for these two types of habitats revealed four clusters based on the Jaccard distance matrix (Supplementary Figure A.3). Permutational MANOVA suggest that both pH (R^2 = 0.099, P < 0.001) and temperature (R^2 = 0.069, P < 0.001) influence the methanogenic β-diversity in nonsaline soils and lake sediments (Table 2.2). Accordingly, we assigned the four clusters to the pH and MAAT of the initial sampling site and obtained largely consistent subgroups to the

community clustering (Figure 2.5C, 2.5D). The combination of environmental characteristics and these four community clusters enables us to define these four subgroups as neutral and cold group1, acidic and cold group2, acidic and moderate group3, and neutral and warm group4 (Figure 2.5). Further PCoA ordination based on the Jaccard dissimilarity matrix suggests that along the PC1 axis, most of the samples from group2 and group3 are from acidic soils and lake sediments while group1 and group4 were mainly from neutral environments (Figure 2.5A). Moreover, samples from moderate sites (group3) separated from those of warm and cold sites along PC2, whereas the samples from the warm environments (group4) separated from the other samples along PC3 (Figure 2.5B). Thereby the first three axes of the PCoA ordination explain 38.8% of the total variation.

Table 2.1 Permutational MANOVA analysis on Jaccard distance matrix of all the samples from the six habitats to test the association of community variance with different environmental variables. The statistical significance with *P* values <0.001, <0.01 and <0.05 were highlighted by '***', '**' and '*', respectively.

Environmental variables	Not subsampled		Subsampled to 15 sequences	
	R^2	*P*	R^2	*P*
Salinity	0.09890	0.0001***	0.09998	0.0001***
Elevation	0.01894	0.0003***	0.02026	0.0158*
Latitude	0.02145	0.0002***	0.03136	0.0001***

Table 2.2 Permutational MANOVA based on a Jaccard distance matrix of nonsaline soil and lake sediment samples to test the association of community variance with different environmental variables. The statistical significance with *P* values <0.001, <0.01 and <0.05 were highlighted by '***', '**' and '*', respectively. MAAT: mean annual air temperature; MAP: mean annual precipitation.

Environmental variable	Not subsampled		Subsampled to 15 sequences	
	R^2	*P*	R^2	*P*
pH	0.0992	0.0001***	0.09334	0.0001***
MAAT	0.0699	0.0001***	0.06342	0.0006***
MAP	0.0447	0.0098**	0.03375	0.2595
Elevation	0.0577	0.0009***	0.04788	0.0211*

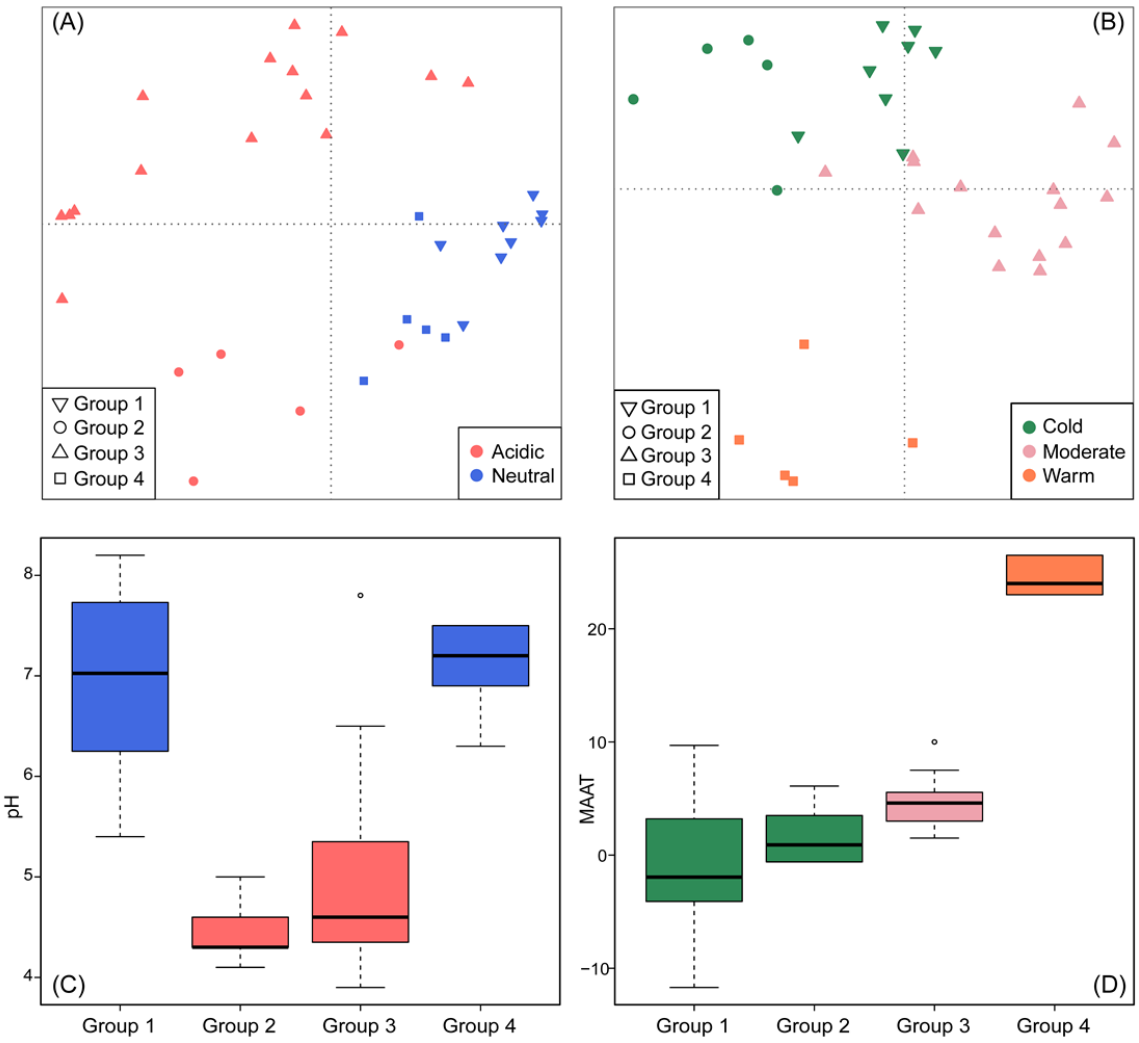


Figure 2.5 PCoA plot based on a Jaccard distance matrix for 33 nonsaline soils and lake sediments. Subplot (A) shows PC1 and PC2 and symbols are colored by pH, and subplot (B) shows PC2 and PC3 and symbols are colored by temperature. The first three components explain 17.1%, 11.7% and 10% of the variance. The box plots show the pH (C) and MAAT (mean annual air temperature) (D) of four identified sub-groups. The box color in figure C corresponds to the pH category in figure A. The colors of the boxplots show statistical significance based on a pairwise Wilcoxon test (P <0.05), where the samples with the same color do not significantly differ from each other. Similarly, color in figure D follows the temperature grouping in figure B. The sub-groups refer to the hierarchical cluster analysis of community similarities.

We examined the occurrence of methanogenic lineages in each subgroup based on incidence frequencies (Figure 2.6). *Methanoregula* prevails in all types of nonsaline habitats. In addition to *Methanoregula*, the neutral and cold subgroup (group1) displays a high abundance of *Methanosaeta*, *Methanobacterium* and *Methanosarcina*. The acidic and cold group2 is represented by *Methanobacterium*, *Methanocella* and *Methanosarcina* while *Methanosaeta* is absent here. *Methanocella* and *Methanosaeta* are common in the acidic and moderate group3. In the neutral and warm group4, *Methanolinea* and *Methanosaeta* are important members. This group is the only group where *Methanoculleus* was identified.

For all four groups, the taxa having a high incidence are *Methanoregula*, *Methanobacterium*, *Methanosarcina*, *Methanosaeta*, *Methanomassiliicoccus* and *Methanocella*. The specialist taxa, which are significantly more represented in most of the sites within a given group, were detected according to indicator species analysis as described before. In total, six out of the 31 taxa showed a significant indicator value ($P < 0.05$) (labeled with asterisk in Figure 2.6). Group1 (neutral and cold) showed the largest number of specialist with lineages of *Methanosaeta*, *Methanolobus* and *Methanomethylovorans*. *Methanobacterium* served as a specialist taxon in the cold and acidic group2 while *Methanolinea* was identified as a specialist in group4 (neutral and warm) but was hardly observed in other groups. In addition, *Methanoregula* is largely represented in the acidic and moderate group3.

2.4.5 Dispersal limitation

A linear regression analysis ($R^2 = 0.05$, $P < 0.001$) indicated a weak correlation between geographical distance and methanogenic community structure on the global dataset. At the same time, a Mantel test showed that the environmental variables have a higher correlation to the community structure than the geographic distances (see Table 2.3). This trend is also confirmed by a partial Mantel test controlling for autocorrelation effects. Plotting the geographical distance against Jaccard community

similarity shows no clear linear trend but patterns which mainly result from the global distribution of the sampling points (see Supplementary Figure A.4).

In order to further analyze the influence of dispersal, we limited our analysis to Europe, which was sampled densest and most even. Mantel tests and partial Mantel tests on this subset reproduced the trend that the community data is higher correlated to the environmental variables than to geographical distances (see Table 2.3). The partial Mantel test controlling for environmental variables could not detect any statistically significant correlation between microbial community and geographic distance. A spatial PCA analysis on these 16 European sites implies a spatial structure of the methanogenic community (23.7% of the total variance was explained by this structure) which corresponds to a positive spatial autocorrelation of the sites as indicated by the Moran's I index (Moran's I = 0.4018). Only the first eigenvalue was stable and corresponded to a separation of the samples between Central Europe and the Baltic States (Supplementary Figure A.5). The small data set, however, complicates a robust assignment of this observed spatial structure to geographical, environmental variables or to both of them. We therefore carried out a cluster analysis on the methanogenic communities as described above and revealed 3 groups which we projected on a geographical map (Figure 2.7A). The clustering did not reproduce the separation of the spatial PCA along the Baltic Sea. Accordingly, some sites which are geographically very close to each other exhibit dissimilar methanogenic community structures and assemble with different clusters. On the other hand, some geographically very distant sites show very similar community compositions and cluster together (Figure 2.7B). The regional dispersal of the groups in Europe does not seem to correspond to or to be limited by a geographic structure.

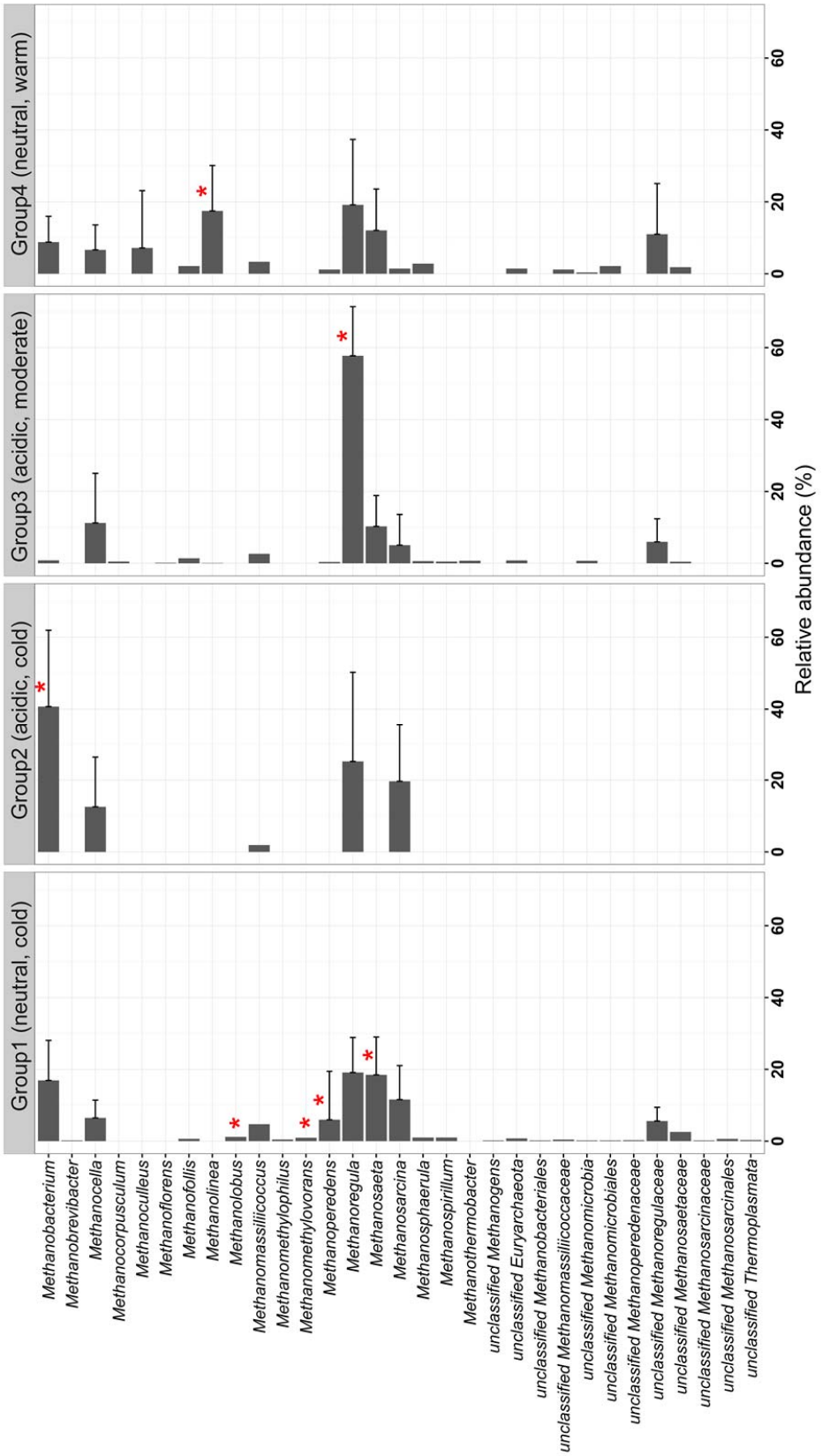


Figure 2.6 Incidence frequencies of methanogenic lineages within the four defined groups of methanogenic communities in soils and lake sediments. The vertical axis is arranged in alphabetic order. The bar length corresponds to the average incidence frequencies for each lineage within the corresponding group. The error bars represent the standard deviation of a given taxon over different samples in that group. Asterisks show the specialist taxa with *P* value < 0.05. The taxonomy is shown for the genus level. If an assignment to the genus level was not possible the next higher assignable taxonomical level was used. The description for the four groups is given in Figure 2.5.

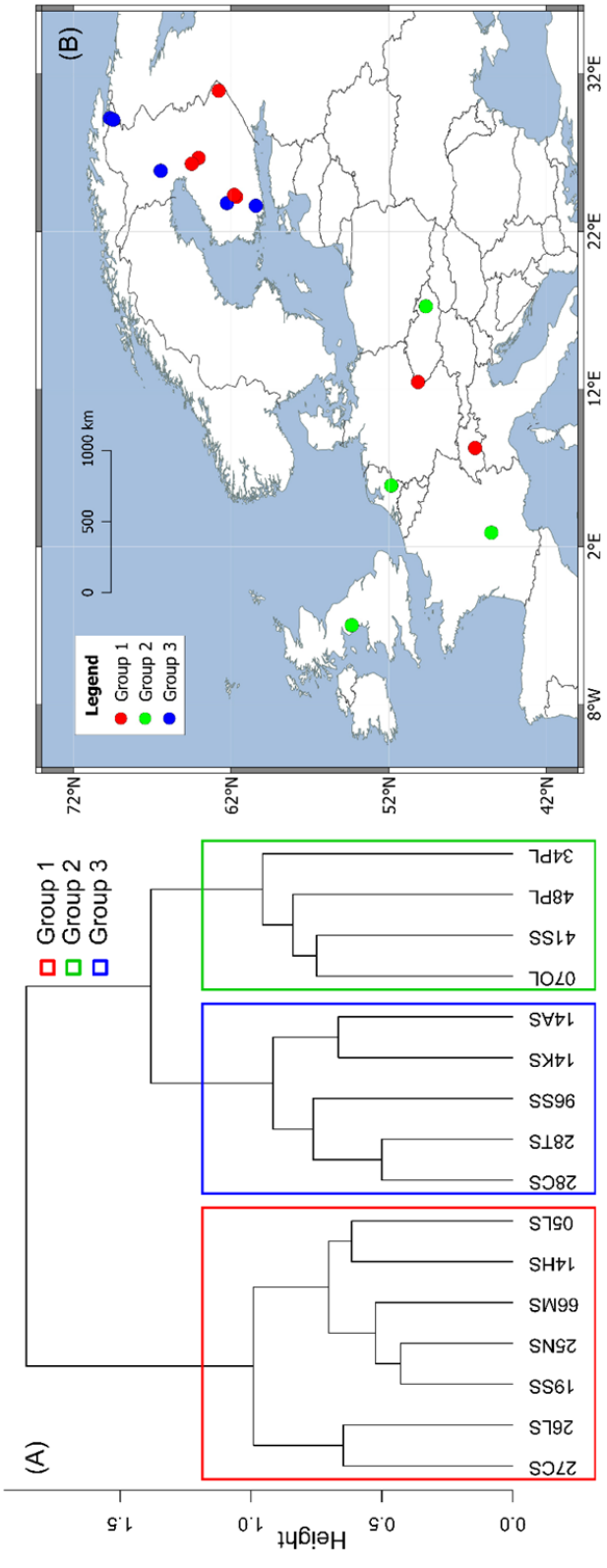


Figure 2.7 Hierarchical cluster analysis of Jaccard distance matrix among 16 European soil and lake sediment samples. The Ward clustering method was used for the analysis. The sites were divided into 3 groups colored by different rectangles in clustering dendrogram (A) and were projected on a European map (B).

Table 2.3 Mantel and partial Mantel test analyses for the determination of the influence of environmental variables and spatial correlation onto the microbial distribution for the global dataset and a subsample of 16 European samples. Geodist: Geographical distance; Envdist: Environmental distance; Jacdist: Jaccard distance.

Test	Matrices	Global samples		European samples	
		r	*P*	*r*	*P*
Mantel	Geodist vs. Jacdist	0.2153	< 0.001	0.3884	0.0032
	Envdist vs. Jacdist	0.3838	< 0.001	0.4421	0.0019
Partial Mantel	Geodist vs. Jacdist (Envdist conditioned)	0.1436	< 0.001	0.0598	0.2685
	Envdist vs. Jacdist (Geodist conditioned)	0.3525	< 0.001	0.2364	0.0380

2.5 Discussion

Identifying and applying concepts of biogeography on microbial communities is of major interest in microbial ecology. Microbial biogeography is believed to be governed by the evolutionary and ecological interplay of four major processes: habitat filtering, dispersal, drift and mutation (Hanson et al 2012). Even though the influence of drift and mutation is beyond the interpretation power of this study, we show that global patterns of methanogenic communities in natural environments exist. This study demonstrates a global biogeographic pattern of methanogenic communities that is more associated with habitat filtering than with geographical dispersal. Methanogenic communities from soda lake sediments, for example, cluster closely with geographically distant marine samples (Figure 2.4) and very similar methanogenic communities occur in European soil and lake sediments despite located in large distance between each other (Figure 2.7). Overall, our attempts to disclose potential dispersal limitation revealed a weak influence of geographic location on methanogenic community structure which contrasts a clear influence of environmental conditions. A conclusive exclusion of spatial effects onto the microbial

communities is not possible with the available data points. Sampling points focus on certain habitats and/or areas while the overall number of samples is low. If there is an effect, we assume spatial effects to occur on regional or local scale only. Local dispersal limitations were reported for hyperthermophilic archaea caused by geographic barriers (Whitaker et al 2003), ammonia-oxidizing bacteria at local salt marshes, but not regional and continental scales (Martiny et al 2011), microorganisms in deep-sea sediments together with environmental settings (Schauer et al 2009), and for bacteria of a large set of heterogeneous snow environments mostly caused by the availability of allochthonous carbon (Lutz et al 2016). A minor influence of dispersal limitation on methanogens in natural environments means that methanogens can randomly distribute over space, are successfully selected by the local environment if their physiological requirements are met and can establish stable communities (Martiny et al 2006, von Mering et al 2007).

The concept of habitat filtering implies that species with similar ecological requirements should co-occur more often than expected by chance (Cornwell et al 2006, Ulrich et al 2010, Weiher and Keddy 2001). Our result show that large differences among methanogenic community composition occur between marine and wetland and lake ecosystems, while estuaries cluster in between. Biogeography patterns based on a set of gene surveys on environmental samples were also reported for general bacteria (Nemergut et al 2011), the methane seep microbiome (Ruff et al 2015), ammonia-oxidizing archaea (Cao et al 2013), marine pelagic and benthic bacteria (Zinger et al 2011), and nitrogen-cycling microorganisms (Church et al 2008) . Habitat filtering was specifically reported for uncultured archaea (Auguet et al 2010), entire bacterial communities in diverse environments (Chaffron et al 2010, von Mering et al 2007) or in South American peatlands at regional scale (Oloo et al 2016), as well as specific bacterial groups such as methane-oxidizing (Knief 2015) and nitrogen-fixing bacteria (Nelson et al 2016) .

Our results indicate that at the global scale salinity substantially regulates methanogenic community composition and determines large differences between

marine and terrestrial methanogenic assemblages. Also methanogens from soda lake sediments cluster with those from marine sediments (Figure 2.4) highlighting the global influence of salinity. This result is in accordance with other studies based on the 16S rRNA gene disclosing that salinity is a primary factor shaping global patterns of overall bacterial and overall archaeal communities (Auguet et al 2010, Cao et al 2013, Caporaso et al 2011, Lozupone and Knight 2007). A low influence of geographical separation but a strong impact of salinity on general microbial communities was also observed in previous studies (Logares et al 2013, Yang et al 2016a). Accordingly, salinity largely determines which lineages can survive. In various habitats methane production activity was negatively correlated with salinity (Bartlett et al 1987, Poffenbarger et al 2011, Potter et al 2009). The inhibition of methane production through salinity is thereby suggested to coincide with a reduced methanogenic population size (Pattnaik et al 2000). The effect of salinity on hydrogenotrophic, acetotrophic and methylotrophic methanogenesis thereby depends on the level of salinity and is different for the different pathways of methanogenesis (Liu et al 2016). Currently, there is no clear mechanism to explain the impact of salinity on community structure but several hypotheses may serve as possible explanation. Physiologically, salinity influences the external and internal osmolarity of cells. The nonsaline methanogenic cells have developed physiological adaptions to counter internal turgor pressure, while the salt-adapted cells have lost such feature (Zinder 1993). In addition, increasing salinity can induce methanogens to synthesize or take up an increased proportion of compatible solutes at a significant energetic and thus metabolic cost (McGenity 2010). The trait of salt tolerance is even manifested in the optimum concentration of NaCl for growth of methanogenic pure cultures since we found that the isolates from marine sediments and hydrothermal sediments have significantly higher optimum NaCl concentration than those from soils (Supplementary Figure A.2).

In the nonsaline terrestrial ecosystems, specifically in soils and lake sediments, the methanogenic community composition is controlled by the combination of

temperature and pH. Accordingly, methanogens of these environments could be classified in four groups (Figure 2.6). Unlike marine ecosystems, the nonsaline terrestrial ecosystems show a large natural variability both of pH and temperature. Temperature can affect not only the methanogenic pathway but also methanogenic populations themselves (Conrad 2007, Rooney-Varga et al 2007). Methane production can be greatly enhanced if temperatures rise as a consequence of the temperature-sensitive steps during fermentation and acetogenesis (Kotsyurbenko et al 2007, Megonigal et al 2004). In addition, low pH can substantially limit the availability of acetate by preventing acetate from dissociating and thus negatively affect acetoclastic methanogenesis (Bridgham et al 2013, Fukuzaki et al 1990). This could be a possible reason that *Methanosaeta* was absent in the group2, while *Methanosarcina* can switch between different sources and was not substantially influenced. Moreover, pH can regulate the efficiency of methane production and methanogenic pathways from ombrotrophic to minerotrophic peatlands, through direct inhibition of both methanogenesis pathways and indirectly through its effects on fermentation (Ye et al 2012). Therefore, both temperature and pH can directly or indirectly regulate metabolic steps associated with methanogenesis and the upstream fermentation, which provides substrate for methanogens.

Methanoregula is ubiquitous and very abundant in all four groups of terrestrial habitats (Figure 2.6) but virtually absent from the marine system and may thus prove to be a proxy for freshwater influence in the marine realm. Its global relevance was recently reported elsewhere (Yang et al 2017). Despite its ubiquitous distribution in soils and lake sediments, *Methanoregula* occurs as an indicator lineage in acidic habitats with moderate temperatures. Moreover, 1) *Methanolinea* appears to have particularly adapted to the warm, neutral terrestrial environments, 2) *Methanobacterium* to the cold, acidic environments, and 3) *Methanosaeta* to pH neutral environments, which is consistent with other studies (Rosenberg et al 2014) and underlines the robustness of our approach. Generally, the geochemical conditions surrounding methanogenic communities will lead to niche differentiation. Since the

niche sorting tends to leave the adaptive specialists (Langenheder and Székely 2011), the progressive long-term environmental selection generated a variety of niches that were filled by an array of endemic habitat specialists, which may be less represented or absent in other different environmental conditions. The community is also shaped by biotic factors, such as ecological interactions, dynamics, competition and symbiosis. Despite those biological factors, Von Mering *et al.* found that habitat preferences are often remarkably stable over time and the distinctive taxonomic composition of environmental communities, in turn, may be an important indicator of their ecology and function (von Mering et al 2007).

Consistent to the habitat preference of methanogenic archaea, it appears that closely-related methanogenic strains were often isolated from comparable environments. For example, *Methanoregulaceae* seems to be quite diverse in natural environments (Yang et al 2017) and neutral strains may resist culturing so far. The currently described strains of *Methanoregula* are both obtained from slightly acidophilic environments, while the two representatives of *Methanolinea* are from relatively warm habitats such as digester sludge and rice field soil, respectively (Rosenberg et al 2014). Although *Methanobacterium* strains were isolated from various environments, approximately half of the existing isolates of this genus exhibit pH optima slightly less than 7. This means that the indicator lineages, which were identified based on the environmental sequences, could reflect the differentiations of physiology and sources of the existing methanogenic cultivars. For example, the habitat salinity, as a general property of habitat, can progressively expose organisms to strong environmental selection and filter the assembly of a new set of species which are best suited for the ambient salinity (Logares et al 2013).

Biodiversity conservation and management is a primary challenge of our current society. Here we show that methanogenic archaea of natural environments are most diverse in estuary sediments. Estuaries are transition zones between marine and terrestrial ecosystems. This allows for two major processes that can contribute to species richness. Firstly, microbes from the sea and land mix at the estuaries and

eventually encompass an overall high diversity (McLusky and Elliott 2004). For example the high diversity of estuary bacteria, archaea, fungi, and even specific bacteria performing unique functions have been observed (Crump et al 2012, Cunliffe et al 2008, Mosier and Francis 2008). Another aspect is the high nutrient level due to the terrestrial, tidal inputs for estuary organisms to feed on (McLusky and Elliott 2004, Statham 2012). In this context, the estuary environments are of importance in recovering generic novelty for methanogens. So far, the effects of species diversity on ecosystem processes have attracted substantial research efforts. The link between biodiversity and ecosystem function is still under debate and remains elusive for microbial communities (Loreau et al 2001, Tilman et al 2014). Even though soils and lake sediments are primary sources of methane and also habitats with high methanogenic diversity, we propose that species richness is not an appropriate proxy of methane production potential and ecosystem methane emissions; it rather seems to reflect the heterogeneity and history of the environment. Ranking the environments according to their species richness does not necessarily mean the potential of methane emission rates which are highest from soils and lakes and comparably minor from estuaries (Figure 2.3 and Supplementary Figure A.1).

Finally, the lack of environmental information in the public databases may have hampered a full interpretation on the environmental drivers observed here. Even though there is mounting sequencing data about methanogens in the literature and public databases, the related abiotic variables provided are often inconsistent and sparse. The limited amount of consistent information for environmental variables does constrain the application of multi-variate statistical analyses. Recalling that the abiotic factors in this study can only explain a limited fraction of the community variances suggests that other explanatory variables are missing. Of particular importance could be the concentrations and the availability of methanogenic substrates such as acetate, hydrogen and methylamines. Nonetheless, abiotic parameters may never suffice to fully explain what structures methanogenic assemblages simply because habitats have different histories and can only be studied

locally. Also, among the available *mcrA* data the vast areas of the Russian and Canadian Subarctic and Arctic are poorly represented. A better geographical coverage and even distribution of *mcrA* gene dataset would improve an assessment of methanogenic communities at a global scale.

3 Predominance of methanogens over methanotrophs contributes to high methane emissions in rewetted fens

3.1 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.

3.2 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 (Abdalla et al 2016, Joosten et al 2015). 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., Emsens et al 2016, Hahn-Schöfl et al 2011, Hahn et al 2015, Jerman et al 2009, Putkinen et al 2018, Urbanová et al 2013, Vanselow-Algan et al 2015, Zak et al 2015), the post-rewetting distribution and abundance of methane-cycling microbes in rewetted fens has seldom been examined (but see Juottonen et al 2012, Putkinen et al 2018, Urbanová et al 2013).

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(Abdalla et al 2016, Segers 1998). 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) (Conrad 2007). 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 (Keller and Bridgham 2007, Knorr

et al 2009, Segers 1998). 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, Juottonen et al 2012, Yrjälä et al 2011), alpine fens (e.g., Cheema et al 2015, Franchini et al 2015, Liebner et al 2012, Urbanová et al 2013), subarctic fens (Liebner et al 2015), and incubation experiments (e.g., Emsens et al 2016, Jerman et al 2009, Knorr et al 2009, Urbanová et al 2011). 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 (Juottonen et al 2012, Komulainen et al 1998, Tuittila et al 2000), 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 (Hahn-Schöfl et al 2011, Hahn et al 2015, Jurasinski et al 2016, Zak and Gelbrecht 2007). 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

$m^{-2} \cdot a^{-1}$ (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 (Minke et al 2016, Urbanová et al 2013). 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 $m^{-2} \cdot a^{-1}$ for pristine peatlands.

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 (Galand et al 2005, Kelly et al 1992), to dominate the methanogenic community in both fens.

3.3 Methods

3.3.1 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 (Figure 3.1a 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 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 (Koebsch et al 2013, Schönfeld-Bockholt et al 2008) 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 kg CH_4 $m^{-2}{\cdot}a^{-1}$ to 0.26 ± 0.06 kg CH_4 $m^{-2}{\cdot}a^{-1}$ (Hahn et al 2015).

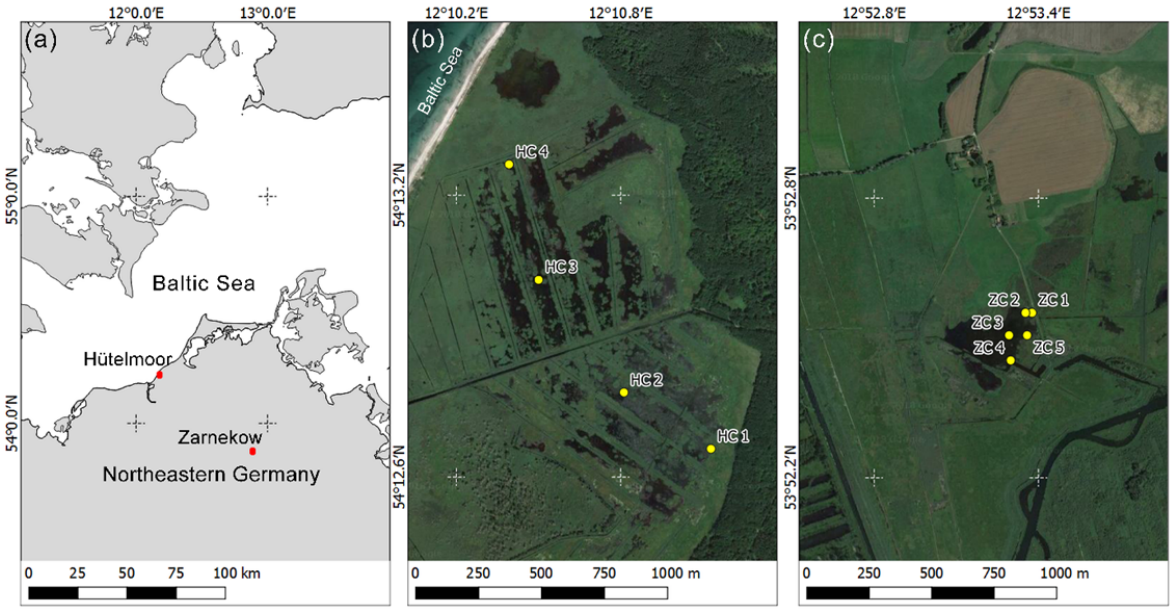


Figure 3.1 Location of study sites in northeastern Germany (a) and sampling locations within sites (b) Hütelmoor and (c) Zarnekow. Maps (b) and (c) are drawn to the same scale. Image source: (a) QGIS; (b) and (c) Google Earth via QGIS OpenLayers Plugin. Imagery date: 9 August 2015.

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, Figure 3.1a 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 eighteenth 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) water table depth increased to 0.1–0.5 m above 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 $m^{-2} \cdot a^{-1}$ (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).

3.3.2 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 1,200 m and 250 m, respectively, to cover the whole lateral extension at each site (Figure 3.1b 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 30-40 cm, 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 was chosen for this study. Peat cores were collected with a Perspex liner (ID: 60 mm, 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 50 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 10 cm 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 40 cm 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 handheld; 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 (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 Ion Pac AS-9-HC 4 column, partly after dilution of the sample. Dissolved metal concentrations were analyzed by 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 hours) 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 was 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 one hour, then incubated stationary at 20°C in the dark. For CH_4 oxidation incubations, 5 g of fresh material and 10 ml of MilliQ water was 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 rate were determined by linear regression of CH_4 concentration over all sampling times.

3.3.3 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 Soil DNA Kit (Roboklon, Berlin, Germany). DNA concentrations were quantified with a Nanophotometer P360 (Implen GmbH, München, DE) 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 45 s and 72 °C for 90 s; a final extension of 10 min 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 was 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). Pre-processed 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 2011) 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 (https://www.arb-silva.de) version 119 (Quast et al 2012). The resulting OTU table was filtered for singletons, 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. Hütelmoor sequence read archive accession numbers are SRR5118134-SRR5118155 for bacterial and SRR5119428-SRR5119449 for archaeal sequences, respectively. Zarnekow accession numbers are SRR6854018-SRR6854033 and SRR6854205-SRR6854220 for bacterial and archaeal sequences, respectively.

3.3.4 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 SybrGreen assays on a Bio-Rad CFX instrument (Bio-Rad, Munich, Germany) with slight modifications after 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 7-cycle-step touchdown starting at 62 °C. The functional methanogenic *mcrA* gene was amplified with the *mlas/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. (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 5

µl 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 (Figure 3.4).

3.3.5 Data visualization and statistical analysis

All data visualization and statistical analysis were done in R (R Core Team 2014). The taxonomic relative abundances across samples were visualized through bubble plots with the R package ggplot2 (Wickham 2016). Differences in microbial community composition were visualized with 2-dimensional non-metric multidimensional scaling (NMDS) based on Bray-Curtis distances. The NMDS ordinations were constructed using R package vegan (Oksanen et al 2013). 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 depthwise trends and assess whether differences in microbial community and abundances among the two fens are related to differences in their respective geochemistry.

3.4 Results

3.4.1 Environmental characteristics and site geochemistry

The two rewetted fens varied substantially in their environmental characteristics (e.g., proximity to the sea) and porewater geochemistry (Figure 3.2, Tables 3.1 and 3.2). EC

was more than three 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 abundant in the upper and lower peat profile in Hütelmoor at ~1.5 to 3.0 mM and ~4 to 20 mM, respectively (Figure 3.2). 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 25 cm depths) were much higher in Hütelmoor than in Zarnekow (Figure 3.2). 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 (Figure 3.1b) – 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 detection limit (0.001mM) (Figure 3.2). 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 $\delta^{13}C\text{-}CH_4$ (Figure 3.2) 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 $\delta^{13}C\text{-}CH_4$ 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).

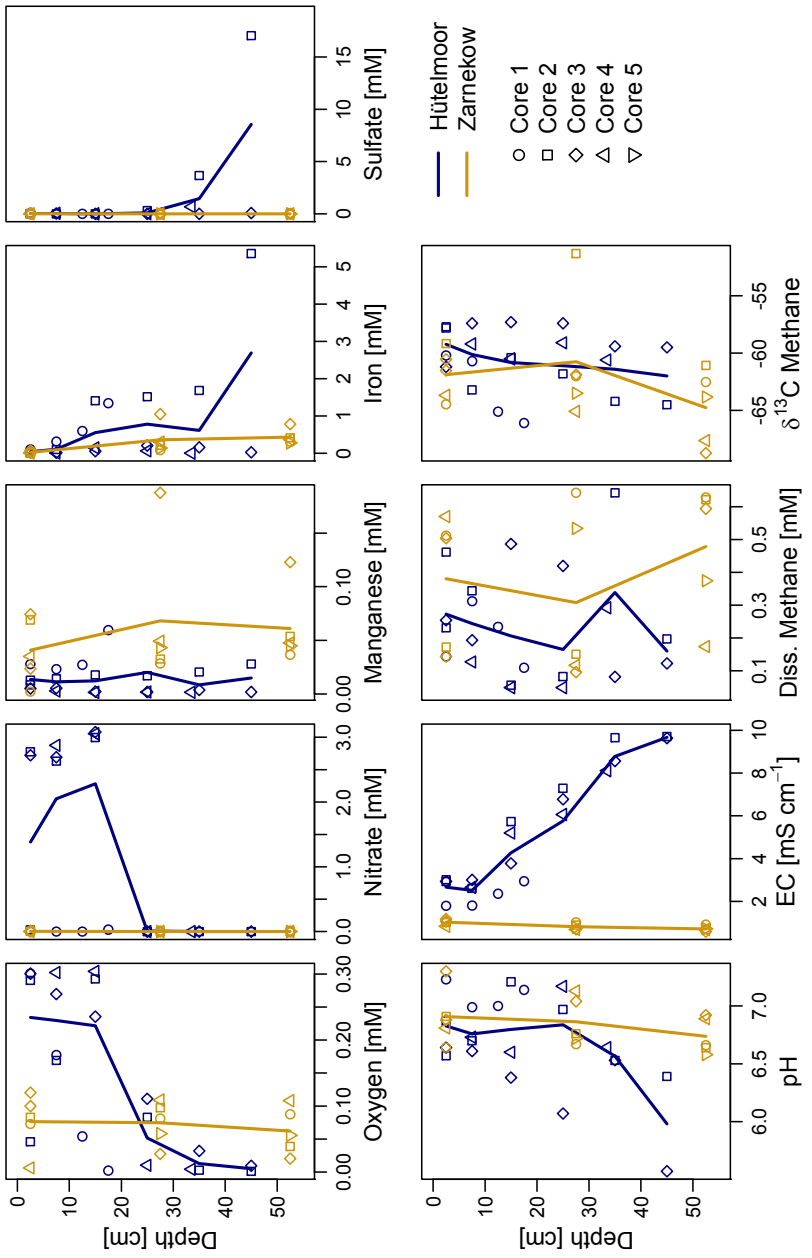


Figure 3.2 Depth profiles of oxygen, nitrate, total iron, manganese, and sulfate (upper panels), and profiles of pH, EC, dissolved methane, and the isotopic signature of methane-bound carbon (lower panels) in both study sites. Solid lines connect the respective means of individual wetlands (n=4 for Hütelmoor and n=5 for Zarnekow).

Table 3.1 Environmental conditions, 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 ($\delta^{13}C–CH_4$), 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	$\delta^{13}C–CH_4$	Dissolved CH_4	O_2	NO_3^-	Fe	Mn	SO_4^{2-}	*16S*	*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^{08}	6.60×10^{06}	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^{07}	6.68×10^{07}	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^{07}	1.76×10^{07}	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^{08}	2.76×10^{07}	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^{07}	1,170
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^{07}	1.69×10^{07}	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^{06}	7.32×10^{06}	0.74
20–30	7.0	7.29	-61.8	0.08	0.08	nd	1.51	0.02	0.29	4.71×10^{09}	6.41×10^{05}	4.50×10^{05}	3.75
30–40	6.5	9.66	-64.2	0.64	nd	nd	1.68	0.02	3.66	2.09×10^{09}	6.21×10^{05}	3.90×10^{04}	18.3
40–50	6.4	9.71	-64.5	0.20	nd	nd	5.35	0.03	17.1	4.09×10^{09}	2.47×10^{06}	2.75×10^{05}	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^{09}	3.51×10^{08}	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^{09}	3.42×10^{07}	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^{08}	9.35×10^{06}	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^{08}	1.92×10^{07}	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^{06}	1.17×10^{06}	2.74
40–50	5.6	9.36	-59.5	0.12	0.01	nd	0.02	nd	0.08	3.18×10^{09}	2.16×10^{06}	2.58×10^{05}	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^{09}	3.09×10^{08}	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^{09}	7.51×10^{07}	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^{08}	2.15×10^{07}	40.8
20–30	7.2	6.06	-59.1	0.05	0.01	nd	0.06	nd	0.02	9.78×10^{09}	5.82×10^{07}	7.91×10^{06}	7.36
30–40	6.6	8.11	-60.6	0.29	nd	nd	0.09	nd	0.67	1.60×10^{09}	1.58×10^{06}	1.25×10^{06}	1.27

Table 3.2 Environmental conditions, 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 ($\delta^{13}C–CH_4$), 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	$\delta^{13}C–CH_4$	Dissolved CH_4	O_2	NO_3^-	Fe	Mn	SO_4^{2-}	16S	*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^{09}	1.49×10^{07}	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^{08}	9.14×10^{06}	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^{08}	6.85×10^{06}	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^{07}	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^{09}	2.34×10^{07}	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^{08}	1.50×10^{07}	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^{09}	3.26×10^{07}	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^{08}	1.55×10^{07}	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^{08}	5.41×10^{06}	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^{09}	6.53×10^{07}	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^{07}	-
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^{08}	4.50×10^{07}	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^{08}	4.97×10^{07}	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^{08}	5.57×10^{07}	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^{08}	1.44×10^{08}	14.9

3.4.2 Community composition of bacteria and archaea

Bacterial sequences could be affiliated into a total of 30 bacterial phyla (Figure 3.3). 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. Within Proteobacteria, the alpha subdivision was the most dominant group, having contributed 26.7% to all the libraries on average (Figure 3.4). 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 (Figure 3.4).

Within the archaeal community, Bathyarchaeota were mostly dominating over Euryarchaeota (Figure 3.5). The MCG group (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 (ZC) 3, ANME-2d represented up to approximately 30% of all archaea but were otherwise low in abundance.

3.4.3 Environmental drivers of microbial community composition

Bacterial and archaeal population at both peatland sites showed distinct clustering (Figure 3.6) 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 (Figure 3.6). Bacterial communities in HC 1 were more similar to communities in all Zarnekow cores than in other Hütelmoor cores (Figure 3.6a). The archaeal community in HC 1 was more similar to Zarnekow cores as well (Figure 3.6b). 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 (Figure 3.6a-c).

3.4.4 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 (Figure 3.7, Tables 3.1 and 3.2). 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 (Figure 3.7). There was a pronounced decrease in microbial abundances at 20 cm depth in the Hütelmoor. For example, 16S rRNA gene

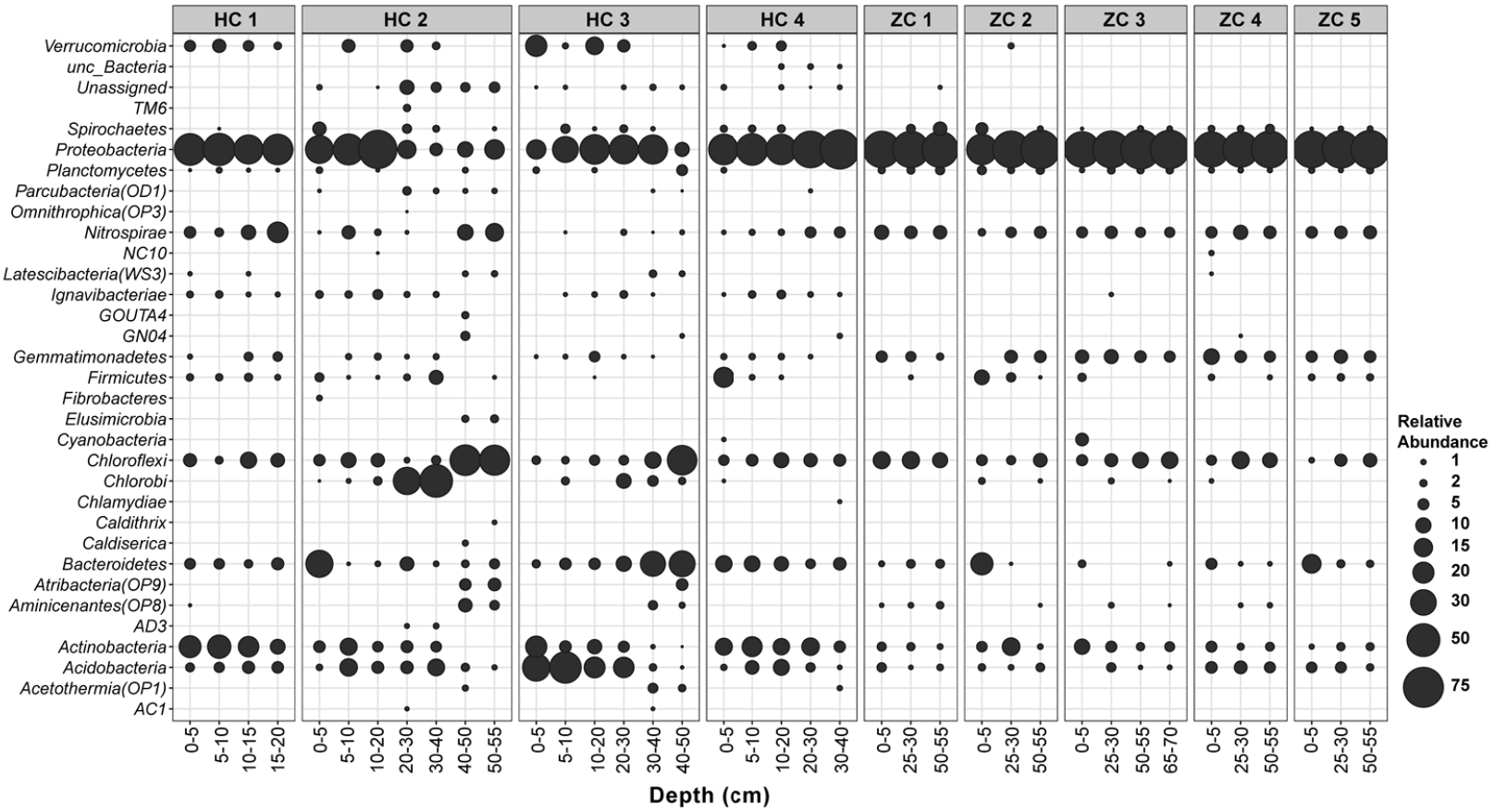


Figure 3.3 Relative abundances of different bacterial lineages in the study sites. Along the horizontal axis samples are arranged according to site and depth. The rank order along the vertical axis is shown for the phylum level.

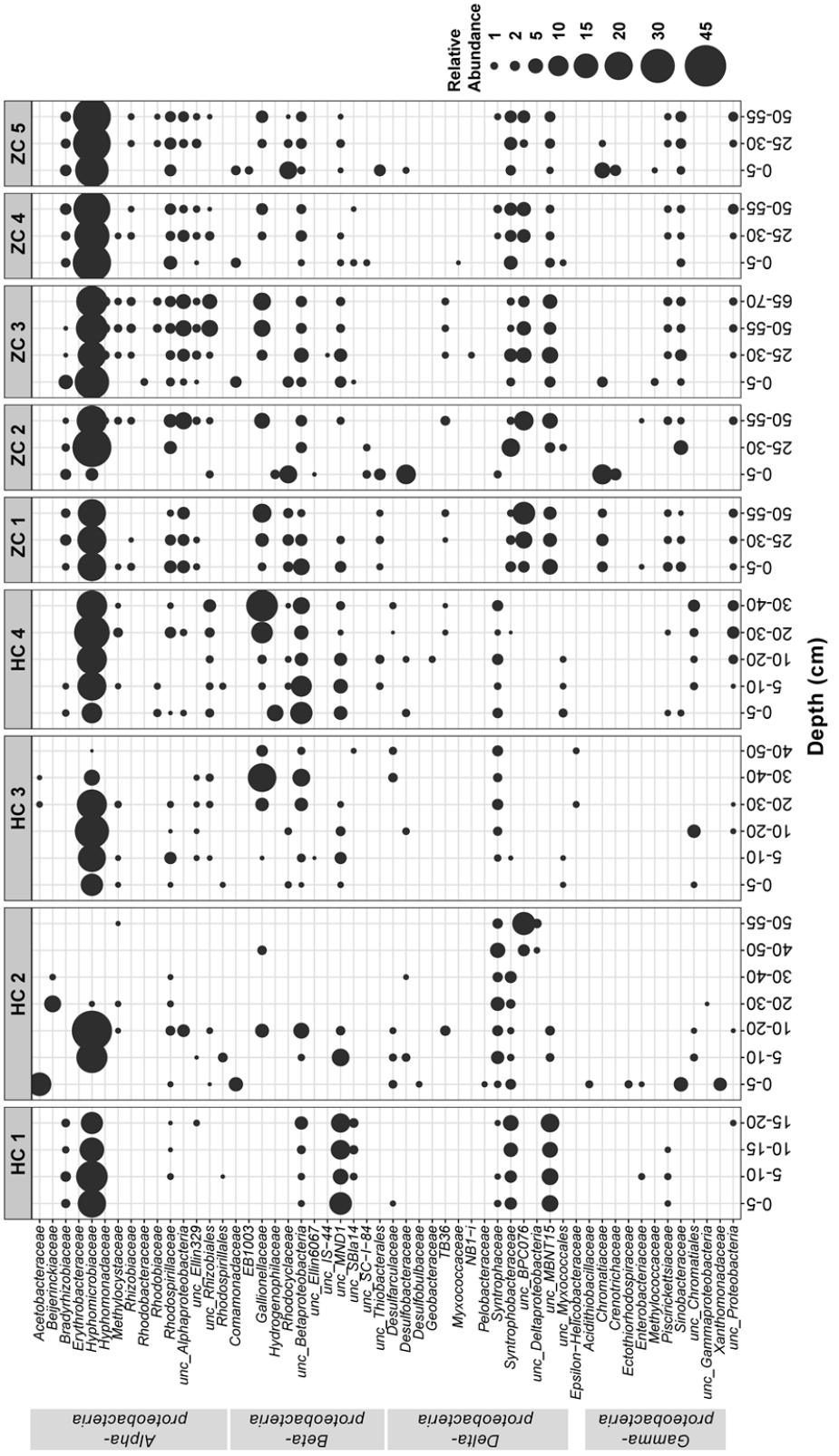


Figure 3.4 Relative abundances within Proteobacteria phylum in the study sites. Along the horizontal axis samples are arranged according to site and depth. The rank order along the vertical axis is shown for the family level. If an assignment to the family level was not possible the next higher assignable taxonomic level was used.

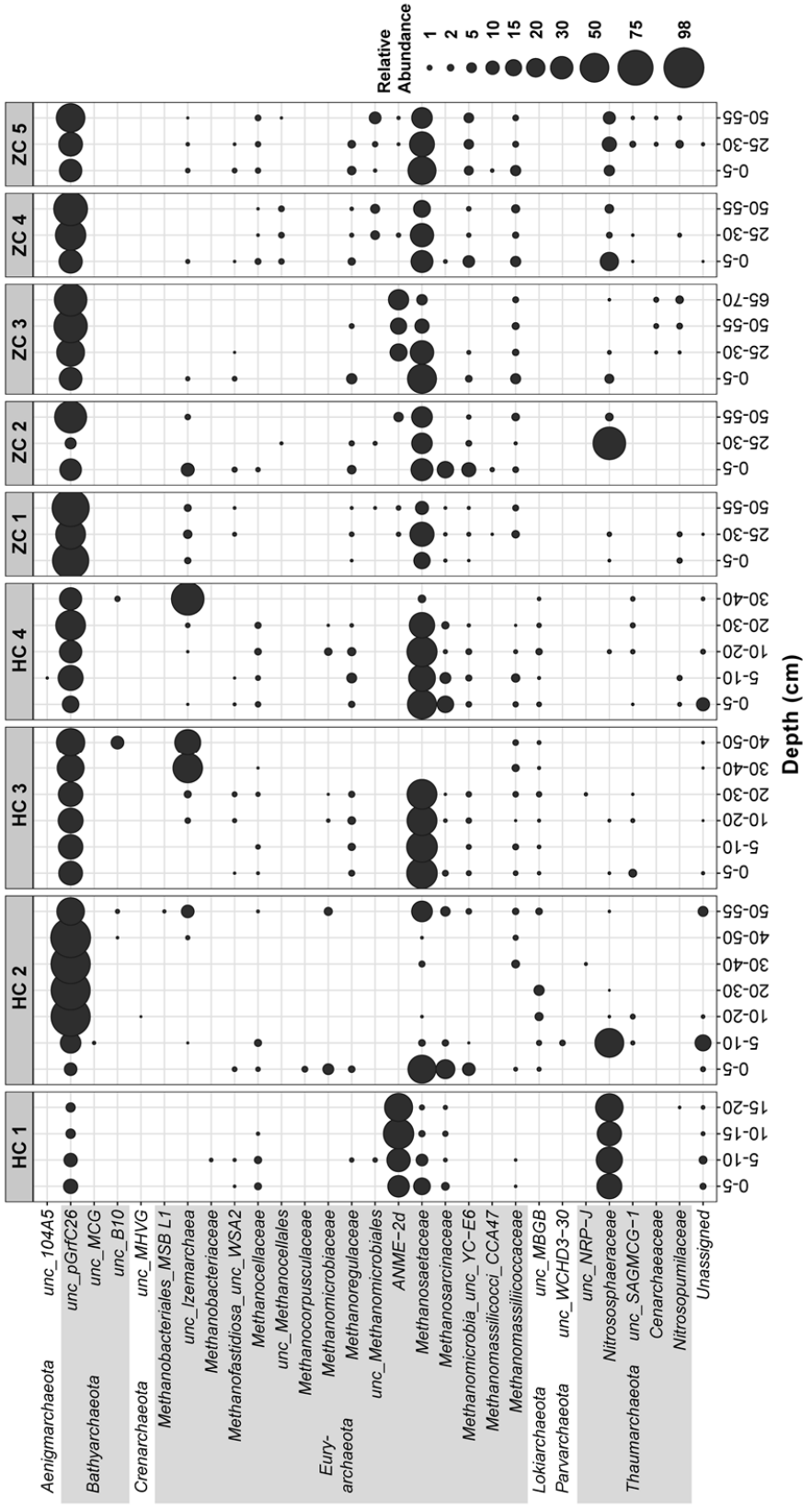


Figure 3.5 Relative abundances of different archaeal lineages in the study sites. Along the horizontal axis samples are arranged according to site and depth. The rank order along the vertical axis is shown for the family level. If an assignment to the family level was not possible, the next higher assignable taxonomic level was used.

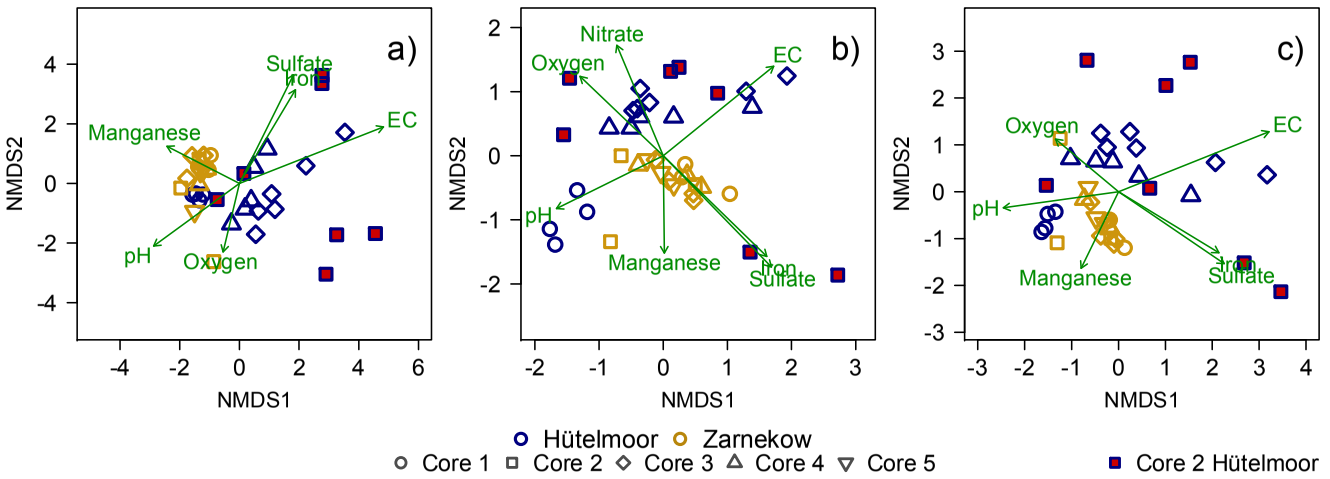


Figure 3.6 NMDS plots showing (a) bacterial, (b) archaeal, and (c) microbial (bacterial plus archaeal) community composition across the nine peat cores. The point positions represent distinct microbial communities, with the border colors of the symbols referring to the study sites and their shapes representing 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.

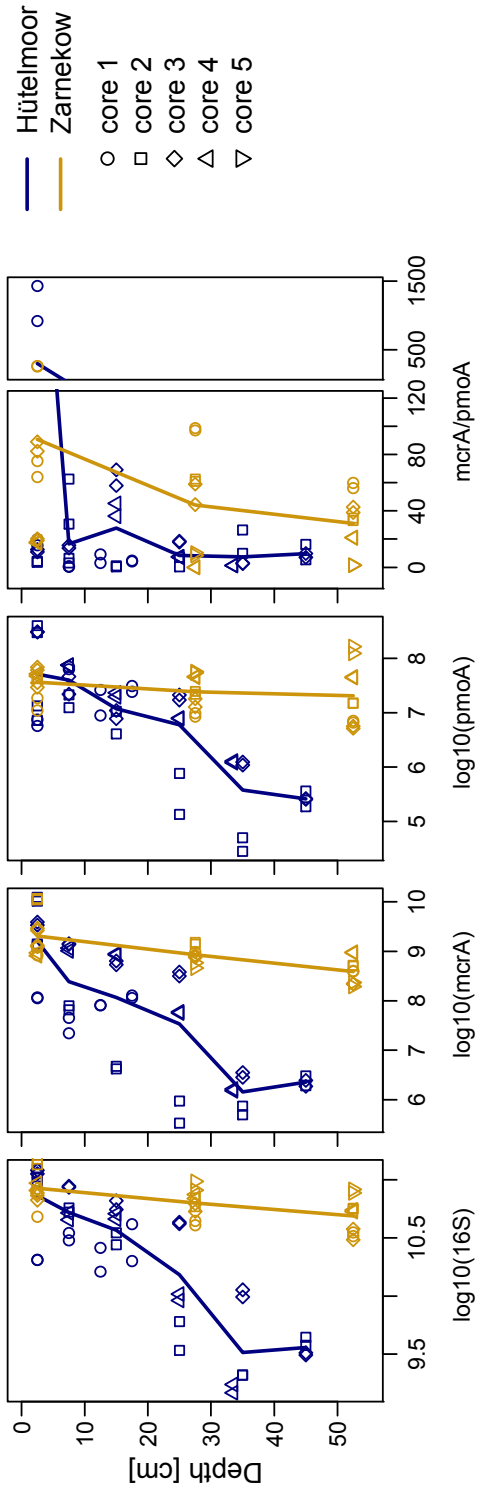


Figure 3.7 Depth distributions of qPCR abundances for total microbial (16S), methanogen (*mcrA*), methanotroph (*pmoA*), and ratio of *mcrA* to *pmoA* gene copy numbers in both sites. Microbial abundances were designated as numbers of gene copies per gram of dry peat soil. Duplicate measurements per 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.

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.

3.5 Discussion

3.5.1 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, Juottonen et al 2012, Yrjälä et al 2011). 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 (Figure 3.2). 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 (Figures 3.2, 3.3, 3.4, 3.6). The NMDS ordinations (Figure 3.6) 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 (Figure 3.6) 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., Wen et al 2017, Yrjälä et al 2011), and TEA availability in peatlands (e.g., He et al 2015).

Comparing the geochemical depth profiles (Figure 3.2) with the relative abundance of bacteria and archaea (Figures 3.3 and 3.4) 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.

3.5.2 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 (Figures 3.3 and 3.4). Functional and ribosomal gene copy numbers not only show a high ratio of methanogen to methanotroph abundance (Figure 3.7), 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 (Cheema et al 2015, Franchini et al 2015, Freitag and Prosser 2009, Freitag et al 2010). 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 (Figure 3.7). 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). 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 (Hernandez et al 2015, 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 (Supplementary Figure A.6) 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., Juottonen et al 2005, Juottonen et al 2012, Yrjälä et al 2011). 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 (Agethen and Knorr 2018, Bridgham et al 2013, Megonigal et al 2004), 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.

3.6 Conclusion

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.

4 Evaluation and update of cutoff values for methanotrophic *pmoA* gene sequences

4.1 Abstract

The functional *pmoA* gene is frequently used to probe the diversity and phylogeny of methane oxidizing bacteria (methanotrophs) in various environments. Here we compared the similarities between the *pmoA* gene and the corresponding 16S rRNA gene sequences of 77 described species covering gamma- and alphaproteobacterial methanotrophs (type I and type II MOB, respectively) as well as methanotrophs from the phylum Verrucomicrobia. We updated and established weighted mean *pmoA* cutoff values on the nucleotide level at 86%, 82%, and 71% corresponding to the 97%, 95%, and 90% similarity of the 16S rRNA gene. Based on these cutoffs, the functional gene fragments can be entirely processed at the nucleotide level throughout software platforms such as Mothur or QIIME which provide a user-friendly and command based alternative to amino-acid based pipelines. Type II methanotrophs are less divergent than type I both with regard to ribosomal and functional gene sequence similarity and GC content. We suggest that this agrees with the theory of different life strategies proposed for type I and type II MOB.

4.2 Introduction

Aerobic methane oxidizing bacteria (MOB), also known as methanotrophs, are commonly grouped into type I and type II MOB belonging to Gammaproteobacteria and Alphaproteobacteria, respectively. Newer studies have uncovered further aerobic and intra-aerobic methanotrophs among the Verrucomicrobia and the NC10 phylum

(Dunfield et al 2007, Nazaries et al 2013). Aerobic methanotrophs catalyze the oxidation of methane through the enzyme methane monooxygenase (MMO) which exists in a soluble (sMMO) and a membrane-bound, particulate form (pMMO) (Nazaries et al 2013). The type I methanotrophs use the ribulose monophosphate (RuMP) pathway to assimilate carbon while the type II utilizes the Serine pathway of carbon assimilation (Trotsenko and Murrell 2008). The alpha subunit of the pMMO, encoded by the *pmoA* gene, is highly conserved (Hakemian and Rosenzweig 2007) and commonly used as a functional gene marker to probe methanotrophs in the environment (Luesken et al 2011). This functional gene marker can simultaneously provide functional and taxonomic records of environmental methanotrophs (Luesken et al 2011) and phylogenetic information as congruent as the 16S rRNA gene (Holmes et al 1995). In practice, the *pmoA* gene fragments were mostly amplified using the forward primer A189f and the reverse primers of A682r (Holmes et al 1995) or mb661 (Costello and Lidstrom 1999).

In the recent years, extensive sequencing efforts on MOB communities have yielded a massive amount of *pmoA* gene sequences from variable habitats. These gene fragments were commonly analyzed after translating them into amino acid sequences. One of the major advantages with amino acid over nucleotide sequences is to guarantee all sequences pass the translation check from the nucleotide to the amino acid sequence. Given all sequences are firstly checked for correct open reading frames (ORF) using tools like FrameBot (http://fungene. cme.msu.edu/FunGenePipeline/ framebot/form.spr), the functional high through-put data could thereafter be further processed at the nucleotide level. In other words, the functional gene fragments could be entirely processed at the nucleotide level throughout software platforms such as Mothur or QIIME which provide a user-friendly and command-based alternative to an amino-acid based pipeline (Figure 4.1). This would also allow calculating distance matrices using Mothur and QIIME which do not yet support amino acids. In this context, precise cutoff values at the nucleotide level are very important for reasonable OTU picking and further community analysis. Moreover, a dedicated reference database of *pmoA* sequences can enable consensus taxonomic assignation for custom

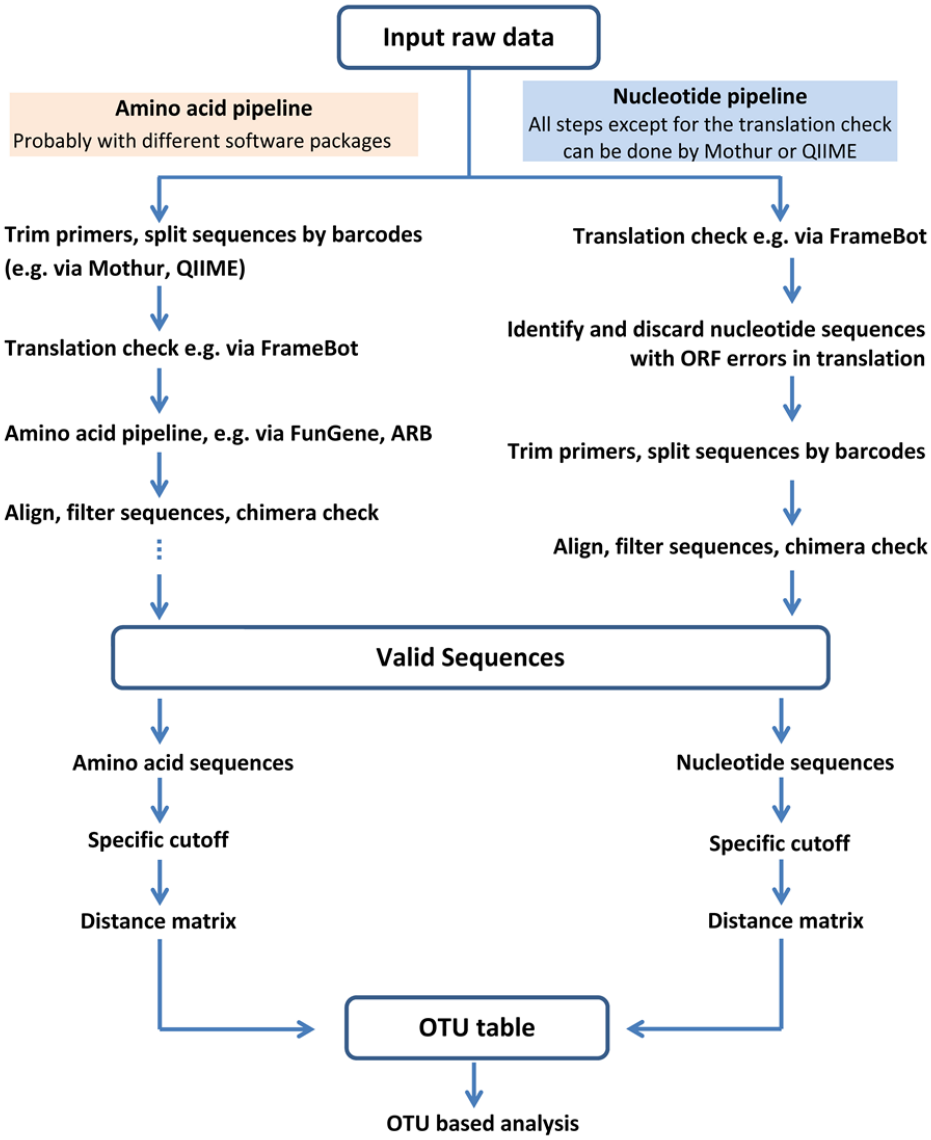


Figure 4.1 Schematic flowchart illustrating pipelines at nucleotide and amino acid levels for processing *pmoA* gene NGS data. Note that except for the very initial step the nucleotide pipeline can be performed throughout the Mothur or QIIME platforms.

data. Dumont and his colleagues have recently proposed a *pmoA* reference database (Dumont et al 2014). From them, the taxonomy is given in a special format which is not as commonly ranked classification schema from phylum to species.

A previous study used 22 methanotrophic isolates and proposed 13% and 7% cutoffs at the nucleotide and protein level, respectively, corresponding to a 3% dissimilarity of the 16S rRNA gene (Degelmann et al 2010). In their study, Degelmann and colleagues provided cutoff values for the species- but not for the genus-level. Another study proposed 10% and 17% dissimilarity of *pmoA* gene nucleotide cutoffs corresponding to 3% and 5% thresholds of the 16S rRNA gene (Lüke et al 2010) by assuming a 3.5 times higher substitution rate (Heyer et al 2002). However, the *pmoA* database has been increasingly expanded with new methanotrophic isolates. Numerous new sequences are not covered by already published *pmoA* gene cutoff values which, therefore, need to be updated. In this study we aimed to evaluate common *pmoA* gene cutoff values at the nucleotide level and to establish such values for the genus and family level taking into account recently isolated methanotrophs as well. The focus is on proteobacterial methanotrophs but Verrucomicrobia related species were also included. We also want to disclose the meaning of individual cutoff values for type I and type II MOB.

Accumulating evidences concerning the ecological characteristics of type I and type II MOB, and community level molecular analyses under different conditions suggest that the different MOB subgroups possess distinct traits (Ho et al 2013). For example, stable isotope labeling experiments demonstrated that type I MOB exhibit significantly higher *pmoA* gene expression level and growth rates than the type II, and are predominantly active in many important habitats with high methane emissions (Chen et al 2007, Dumont et al 2011, Graef et al 2011, Ho et al 2013, Kip et al 2010, Qiu et al 2008, Zheng et al 2008, Zheng et al 2010, Zheng et al 2012). On the other hand, the type II MOB population is relatively stable and serves as microbial seed bank in the soil (Eller et al 2005, Krause et al 2012). These different traits are explained by different r/k strategies of type I and II MOB (Bodelier et al 2012, Ho et al 2013). The sum of these studies renders it convincing that these two strategies exist among methanotrophs. Based on the sequence information necessary for the primary goal of this study we want to compact this theory. If type I and type II MOB possessed different life strategies, this should be reflected in the *pmoA* gene's GC (guanine-

cytosine) content of type I and type II methanotrophs because the GC content is hypothesized as one of the genomic traits that relates with the variation in selection and mutational bias (Birdsell 2002). An additional, minor objective of this work is therefore to compare the *pmoA* gene sequences of type I and type II MOB with regard to their GC content.

4.3 Material and methods

A total of 516 *pmoA* nucleotide sequences of pure cultures were originally retrieved from the FunGene database (http://fungene.cme.msu.edu). Then the corresponding 16S rRNA sequences were sequentially searched from the NCBI database by using a perl script. After removing short and low quality sequences both for the *pmoA* and the 16S rRNA genes, we revealed a total of 77 species (for details, please refer to supplementary Table A.4 and Figure A.7/Table S1 and Fig. S1). These species could be assigned to 19 genera within all known classes of methanotrophs. Two filamentous methanotrophs (fMOB) *Clonothrix* and *Crenothrix* were also included together with three other *pmo*-like (*pxmA*) gene sequences. The 16S rRNA and *pmoA* sequences were aligned against the silva reference file (v119) by using Mothur platform (Schloss et al 2009) and the *pmoA* sequences were aligned with pre-aligned *pmoA* sequences provided by the Fungene pipeline database. Afterwards, the distance matrices were calculated by the R package of ape (v3.3) (Paradis et al 2004) for both the 16S rRNA and the functional *pmoA* gene sequences. The distances were plotted pairwise against each other using the R package of ggplot2 v. 0.9.3.1 (Wickham 2009). The linear and quadratic regressions were calculated by functions provided by the basic package in R (R Core Team 2014). In addition, the regression cutoff values were further evaluated by counting the agreement of both 16S rRNA genes and *pmoA* genes (Figure 4.2 B-D/Fig 2. B-D). Comparing to these ideal values helps to estimate how confident the regression derived values derived are. Finally, all 16S and *pmoA* nucleotide sequences in this study were subject to calculating the GC content by using Biopython script. A pairwise plot of GC content between each 16S and *pmoA* sequences were generated

by using basic packages in R. Besides, we created a more comprehensive taxonomy database for the *pmoA* sequences which could be probed by the primer set combination of A189f and A682r. Sequences in this database were firstly retrieved from the NCBI database and progressively screened by Biopython or R scripts. The corresponding taxonomy was generally referred to the NCBI taxonomy if the taxonomic ranks from phylum to species are available. For those with ambiguous taxonomies given by the NCBI database, efforts have been made to blast against the Dumont's database (Dumont et al 2014) to improve the taxonomic classification as appropriate.

4.4 Results

Briefly, the taxonomic database consists of 7809 unaligned *pmoA* nucleotide sequences of methanotrophs in fasta format and corresponding taxonomy files special for Mothur and QIIME. This database included unique functional sequences of *pmoA* and related (*pxmA*) genes in the methanotrophs within the phyla of Proteobateria and Verrucomicrobia. This library also contains some related *amoA* sequences which are frequently co-amplified by the above mentioned primer set. The taxonomic file is a two column tabular file, with the first column containing the sequence accession number and the second showing the taxonomic information, where the taxonomic levels are separated by semicolons. The database is published as supplementary data to this article and can be accessed via http://dx.doi.org/10.5880/GFZ.5.3.2016.001 (Yang et al 2016b). These files are suitable for assigning sequences to the taxonomy outlines using open-source software such as Mothur and QIIME.

To determine the family, genus and species cutoff values for the *pmoA* gene we referred to the common thresholds of 90%, 95% and 97% sequence identities of 16S rRNA genes, respectively. Based on a linear correlation of the pairwise distances (R^2 = 0.7603), the thresholds of 74.44%, 82.06% and 85.10% were derived for *pmoA*. However, the quadratic fitting analysis which resulted in cutoff values of 70.95%, 81.20% and 86.18%, appears more appropriate (R^2 = 0.8047) (Figure 4.2A). Since all

cutoff values contain a certain degree of arbitrariness, we systematically investigated how well the classification based on the 16S rRNA gene and the *pmoA* gene agrees for different threshold calculations, shown in Figures 4.2B, C and D. The intersections from them suggest *pmoA* gene thresholds of 69.92%, 83.10% and 87.22% according to 16S rRNA gene cutoff values of 90%, 95%, and 97%. Ideally, the *pmoA* gene cutoff values should coincide with the maximum fraction of pairs classified based on both genes. Therefore, the statistical cutoff values by quadratic rather than the linear fitting (Figure 4.2A) are again more preferable. In this regard, it is plausible to set the *pmoA* nucleotide cutoff values at 86% for the species, 82% for the genus, and 71% for the family level (Table 4.1).

Table 4.1 Cutoff values for *pmoA* nucleotide sequences

	Species	Genus	Family	Reference
pmoA	87%	-	-	Degelmann et al. 2010
pmoA	90%	83%	-	Lüke et al. 2010
pmoA	86%	82%	71%	This study
type I MOB	86%	82%	-	This study
type II MOB	87%	82%	-	This study

Because type I and type II methanotrophs exhibit different variability in sequence similarity (inset Figure 4.2), the cutoff values of *pmoA* gene were tentatively computed for the type I and type II, respectively. For the type I methanotrophs, if including the 92 outliers scattering in the bottom right of the plot, the 78% and 83.95% identity of *pmoA* gene fragment roughly correspond to 95% and 97% similarity of 16S rRNA gene. However, the R^2 of 0.2224 indicate a relatively low representative of these values. Excluding the minor part of outliers from the total caused by *Crenothrix* and *Methylococcaceae bacterium* M200 derived sequences substantially improves the fitting goodness (R^2 = 0.5359), leading to the corresponding cutoff values of 82.04% and 86.30% (Supplementary Figure A.8). For the type II MOB, the thresholds are 82.13% and 87.19% equivalent to 95% and 97% of similarity of the 16S rDNA

(Supplementary Figure A.9). Thus, the overall cutoff is very robust and can also individually be applied for type I and type II MOB with a slight underestimation of type II species richness using the general cutoff.

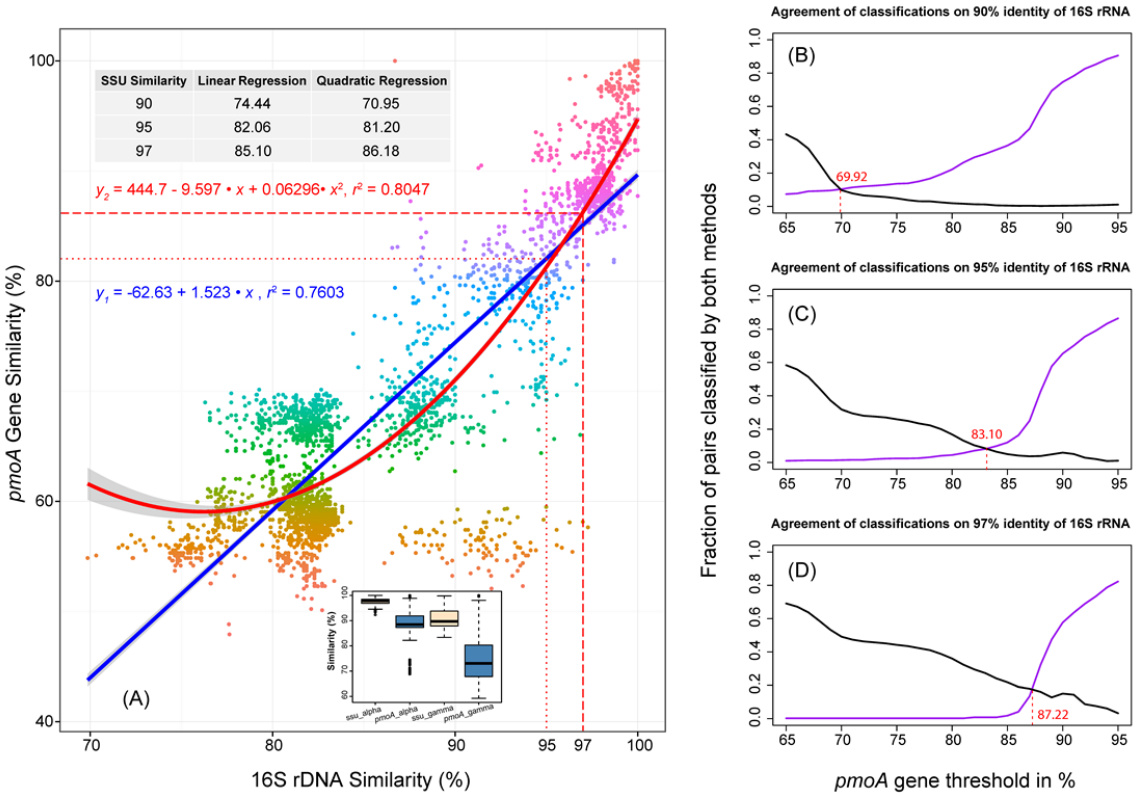


Figure 4.2 Pairwise correlation of similarities based on the 16S rRNA gene versus the *pmoA* gene. The color of the dots in **a** indicates the numerical range of *pmo*A gene similarity. The polynomial and linear fiting lines in **a** are in red and blue, respectively. The inset boxplot describes the quantile statistic of similarities of 16S rRNA and *pmoA* genes of type I (gammaproteobacterial) and type II (alphaproteobacterial) methanotrophs. ANOVA test displayed statistical signifiance in sequence similarities between the two genes ($p < 0.001$). In **b**, **c**, **d** fied cutoff values for the 16S rRNA gene are assumed at 90, 95, and 97% sequence identity while the functional *pmoA* gene threshold is variable. The purple lines show the fraction of pairs classifid on the *pmoA* gene level. Evidently, for a low functional threshold this ratio is 1, while for large values it drops to zero. Conversely, the black lines show the fraction of pairs classifid on the 16S rRNA gene level. This value increases to 1 for large functional thresholds. For the inset boxplot in **a**, the labels of SSU, alpha and gamma stand for the 16S rRNA gene, type II (alphaproteobacterial) and type I (gammaproteobacterial) methanotrophs, respectively.

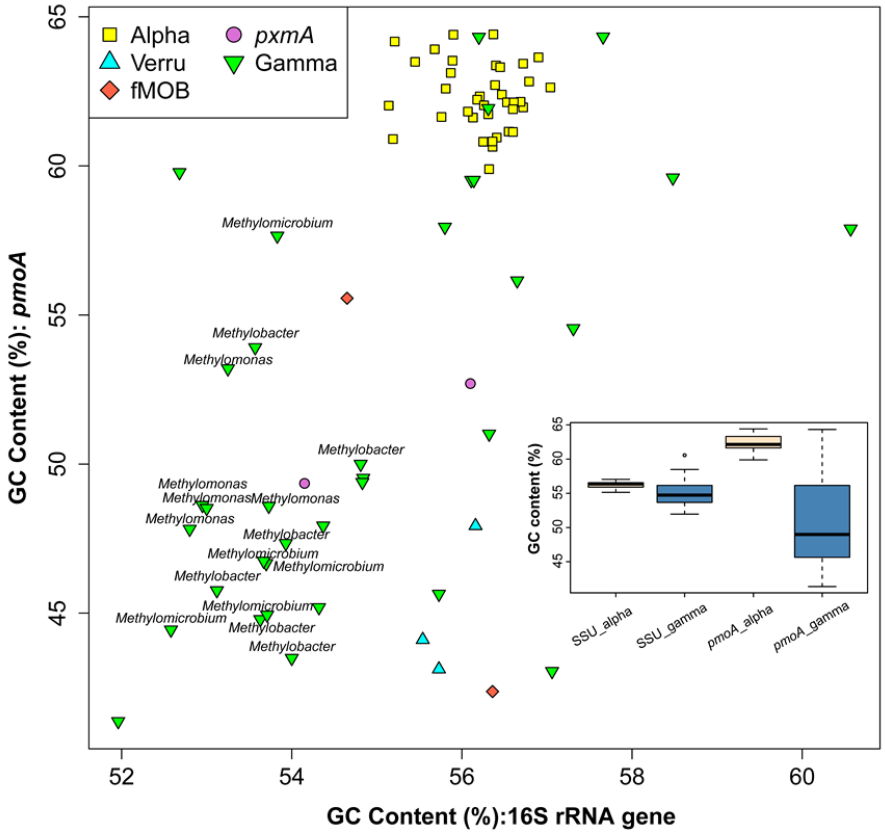


Figure 4.3 Pairwise scatter plot of GC contents between the functional and 16S rRNA gene fragments across MOB used in this study. Specified symbols are used to discriminate different subgroups of methanotrophs (Alpha: type II MOB; Gamma: type I MOB; *pxmA*: methanotrophs with *pxmA* gene fragment; fMOB: filamentous methanotrophic *Crenothrix polyspora* and *Clonothrix fusca*; Verru: Verrucomicrobia). The inset boxplot illustrates the statistical quantiles of GC content of the 16S rRNA gene and the *pmoA* gene fragments.

The GC content of the *pmoA* gene fragments used in this study showed high variations among type I MOB while they only displayed a narrow range among type II MOB which also had a generally higher GC content (Figure 4.3). The narrow range of GC values may partly be biased by the uneven distribution of species per genotype. There are, for example, 22 species belonging to *Methylocystis* (Supplementary Figure A.7),

and they had to be expected to have similar GC values. However, species of genotypes *Methylobacter* and *Methylomonas* (type I MOB) vary greatly with regard to their *pmoA* gene's GC values despite their affiliation to the same genus.

4.5 Discussion

According to our results, the *pmoA* gene cutoff value (14% dissimilarity) for species is 4.7 times higher than the 3% cutoff for 16S rRNA gene, and modifies the Degelmann cutoff (13%) by 1%. Our substitution rate is higher than the previously published rate of 3.5 for partial *pmoA* genes of type II MOB (Heyer et al 2002). The regression of the pairwise plot could cover most of the species (R^2 = 0.8047). The residuals are mainly due to *Crenothrix* and *Methylococcaceae bacterium* M200, which scattered as outliers in Figure 2. *Crenothrix* belongs to the gammaproteobacterial methanotrophs in terms of the 16S rRNA gene, but it shows a very divergent *pmoA* gene (Stoecker et al 2006). The strain M200 shows highest homology on the 16S rRNA gene with various uncultured bacteria from different ecosystems, but it shares only 71-72% homology on the functional gene level with its closest relatives *Methylobacter marinus* and *Methylomicrobium album* (Kip et al 2011, Tavormina et al 2011). Moreover, the *pxm* operon has a different origin and the order of genes is uniquely organized in the non-canonical form different to that in all reported *amo* and *pmo* operons (Tavormina et al 2011). These two species also contributed the major outliers in the pairwise plot of type I methanotrophs (Supplementary Figure A.8). Among the type II methanotrophs, the outliers are mainly caused by *Methylocapsa acidiphila*, which forms a deep divergent clade to the other alphaproteobacteria methanotrophs (Degelmann et al 2010).

The cutoff values based on the regression model are a weighted mean for the two groups. Since the type II MOB have higher similarities and smaller variability than the type I MOB, the tradeoff thresholds should be a bit lower than the actual ones for the

type II MOB while they should be slightly higher for the major type I MOB. The individual cutoff values for type II and type I reflect this difference (Supplementary Figure A.8, A.9), although the regression fitness is relatively small for both groups. As a consequence, assigning OTUs based on the generalized cutoff values lead to slightly underestimated species richness for type II and slightly inflated diversity for type I MOB. Despite these minor limitations, the proposed cutoff values cover the majority of methanotrophic species and represent the most reliable values to date. They allow for a more precise estimation of methanotrophic diversity in the environment.

We have illustrated that the *pmoA* gene's GC content of the type II MOB is generally higher than that of type I MOB and show a rather narrower variability (Figure 4.3). As mentioned earlier, the GC content is suggested to be a genomic trait that relates for example with the variation in selection (Birdsell 2002). Although the hypothesis that the GC content plays a vital role in temperature adaptation has been refuted (Hurst and Merchant 2001), a recent gene-centric association analysis demonstrated that correlation exists at least for certain genomic regions (Zheng and Wu 2010). Experimental evidence shows that the dormant type II MOB can become metabolically active with higher methane uptake rates in response to an exposure to elevated temperatures (Ho and Frenzel 2012, Whittenbury et al 1970), suggesting the high-GC type II can positively respond to higher temperatures. Type I MOB are active in various environments with high methane emissions, while type II MOB are relatively stable and are assumed to be present often in dormant states (Ho et al 2013). Type II MOB also exhibited slower growth rates reflected in lower mRNA transcripts per cell (Steenbergh et al 2010). However, some types II MOBs are less dependent on the availability of other nutrients besides methane (Steenbergh et al 2010). Some facultative type II MOB are able to utilize more versatile substrates than the type I (Ho et al 2013). Therefore, in some case, the type II MOB, although largely dormant, could ultimately dominate the total MOB population following disturbance as type I adversely response to the disturbance (Ho et al 2011). These different features and life strategy give them a survival advantage. In the long process of evolution, type II

MOB, being slow-growing as well as capable of dormancy under unfavorable conditions, tend to propagate to a much lower extent compared to type I populations, and may simultaneously have accumulated fewer mutations in the genomic sequences. This could have possibly also had an effect in producing the differences in GC content we described.

5 Synthesis and conclusion

5.1 Introduction

Methanogenic archaea and methanotrophic bacteria, as the biological producers and consumers of methane, are key players of the methane budget. They have been studied in various environments, but mainly at limited spatial scales or within single habitats. A biogeographic study of methane-cycling microbial communities including defining their main driving forces, both on a local and global scale, would increase our understanding of these functional microorganisms and their distribution patterns in natural environments and restored wetlands. Furthermore, it would provide valuable information in predicting their response and feedback in future climate change.

In this thesis, meta-analysis (global scale) and site-specific analysis (local scale) were conducted to explore the biogeographic pattern of methane-cycling microbial communities, and to investigate the extent to which this pattern is due to environmental selection or dispersal limitation. Environmental parameters regulating methane-cycling communities at multiple scales were also determined. The following synthesis summarizes the main outcomes of this study; the scientific evidence is given in three manuscripts (Wen et al 2016, Wen et al 2017, Wen et al 2018) which are presented in chapter 2, 3, and 4. The questions raised by this study and possible future research directions are addressed in the outlook.

5.2 Distribution of methane-cycling microorganisms in natural environments and restored wetlands

The microbial communities involved in the methane cycle have been explored in oceans, mud, marshes, subsurface environments, soils, rice paddies and landfills (e.g., Angel et al 2012, Cheema et al 2015, Christiansen et al 2014, Großkopf et al 1998, Hallam et al 2007). These studies largely expanded our recognitions of these communities/groups. Molecular methods have allowed researchers to estimate the diversity and abundance of methane-cycling microorganisms from various environmental samples and yielded a large amount of sequences available for meta-analysis at larger spatial scales. For both methanogens and methanotrophs, targeting of the functional marker genes (*mcrA* and *pmoA*) provide alternative and robust signatures additional to the 16S rRNA gene. The 16S rRNA gene sequences were generally processed on nucleotide level, while the gene fragments generated from functional *mcrA* or *pmoA* gene sequencing were often analyzed after translating into amino acid sequences. Using nucleotide sequence information of functional genes directly captures differences of diversity more subtle than the translated amino acid sequences. In addition, nucleotide sequences can be directly processed with popular sequence processing platforms for phylogeny and distance matrix based analysis. To get reliable OTU assignment on the nucleotide level, reasonable and precise cutoff values are crucial. For the 16S rRNA gene it is commonly accepted that the values of 90%, 95% and 97% similarity are used to determine whether the taxa belong to the same family, genus or species. Yang et al. (2014) proposed cutoff values for *mcrA* nucleotide sequences at different taxonomic levels. For *pmoA* gene sequences, however, the cutoff values for family, genus and species levels need to be evaluated and updated as many new sequences are not well represented by the previous cutoff values. For example the cutoff at species level of 87% by Degelmann et al (2010) is different from the threshold of 90% defined by Lüke et al. (2010) probably because those values were derived from different subsets of methanotrophs within which some groups of methanotrophs are less represented.

Therefore, one purpose of this thesis is to update and verify the weighted mean *pmoA* gene cutoff values on the nucleotide level taking into account recently isolated methanotrophs. Corresponding to the 97, 95 and 90% similarity of the 16S rRNA gene, the *pmoA* nucleotide cutoff values are updated to be 86% for the species, 82% for the genus and 71% for the family level, respectively (Wen et al 2016). The proposed cutoff values cover the majority of described methanotrophic species. Our *pmoA* gene cutoff value for species level (14% dissimilarity) modifies the cutoff proposed by Degelmann et al (13% dissimilarity, 2010) by 1%, and the former proposed cutoff value of genus level by Lüke et al (17% dissimilarity, 2010) was updated to 18%. Along with the cutoff modification, a database of methanotrophic *pmoA* gene nucleotide sequences is also constructed, which provide taxonomy files according to the formats of Mothur and QIIME pipelines. With the deliberations on possible nucleotide cutoff values and the presented taxonomic database, this work aims to facilitate research in more consistent estimation of methanotrophic diversity for individual studies. The later built up of methanotrophic studies with consistent cutoff and reference database will provide robust metadata for methanotrophic biogeography studies.

Microbial biogeography examines the spatial distribution of microbial taxa at different spatial scales (Ramette and Tiedje 2007). The biogeographic patterns over different spatial scales have been observed in a wide range of microorganisms such as uncultured archaea, ammonia-oxidizing archaea, and bacteria (e.g., Auguet et al 2010, Cao et al 2013, Hanson et al 2012, Lozupone et al 2007, Martiny et al 2006, Ramette and Tiedje 2007). The biogeography of methanogens at different spatial scales was so far not directly targeted. The next generation sequencing revealed the ubiquitous distribution of methane-cycling organisms and has recently contributed to an exponential increase of methanogenic sequences. With the wealth of the abundant sequences, 94 globally distributed *mcrA* gene datasets together with local datasets from two rewetted fens are examined to detect the biogeographic patterns of methanogenic archaea at different spatial scales.

This thesis for the first time draws a global biogeographic distribution map of methanogenic archaea in six categories of natural habitats, namely, soil, lake sediment, estuary, mud volcano, hydrothermal sediment and marine sediment. The study shows that global patterns of methanogens exist, although some taxa seem to be cosmopolitan (Wen et al 2017). The richness of methanogenic archaea varies largely between different habitat types. Terrestrial habitats (soils, lake sediments and estuaries) in general maintain higher methanogenic diversity than marine habitats (marine sediments, hydrothermal sediments and mud volcanos). Estuaries, which are the connection of terrestrial and marine habitats, contain the largest reservoirs of methanogenic diversity (Wen et al 2017). Coincidently, a meta-analysis study on ammonia-oxidizing archaea (AOA) also demonstrated estuaries as the largest reservoirs for AOA at global scale (Cao et al 2013). Estuaries, therefore, might be promising environments for recovering generic novelty for methanogens and other microorganisms. In addition, the thesis shows preferences of specific methanogenic taxa for certain environmets. The genus *Methanoculleus* is more frequently observed in marine and estuary environments, but rarely detected in soils and lake sediments. In nonsaline soils and lake sediments, *Methanoculleus* is only identified in warmer and pH neutral sites. In addition to *mcrA* fingerprints, *Methanoculleus* isolates were previously retrieved mainly from marine or brackish water, and were found to be mesophiles and thermophiles (Aharon 2014, Surakasi et al 2007). *Methanolinea*, which is identified as indicator spiecies for warm and pH neutral soils and lake sediments, is almost absent in other nonsaline samples. The isolates of *Methanolinea*, which have been retrieved from a wide variety of anoxic environments, prefer optimum temperature range between 37 and 50°C and optimum neutral pH for growth (Imachi and Sakai 2016). Moreover, some methanogenic lineages, including *Methanocaldocccus*, *Methanothermococcus*, *Methanopyrus*, *Methanotorris* and *Methermicoccus* are only detected in hydrothermal sediments. A global meta-study of archaea by Auguet et al (2010) also found that hydrothermal vents held a high number of archaeal indicator lineages. These results indicate that distinct ecosystems

like hydrothermal sediment may be represented by a group of endemic species, therefore, more research efforts are encouraged in those environments.

The global data reveals that *Methanoregula* is the most frequently observed lineage in soils and lake sediments while it is almost absent in marine environments (Wen et al 2017). A dominance of *Methanoregula* in terrestrial ecosystems does not correlated with our fine-resolution data from two local rewetted fens. In both fens, *Methanoregula* displayed a low abundance, especially in the Hütelmoor site which was historically affected by the Baltic Sea (Wen et al 2018), which would support that *Methanoregula* is a freshwater taxon and potentially indicative for a lack of marine influences. The different primer sets might slightly contribute to the inconsistent prevalence of *Methanoregula* between global (*mcrA*-based) and local (16S rRNA gene-based) scales. In addition, the indicator species analysis of the global data suggests that members of *Methanoregula* have particularly adapted to acidic habitats with moderate temperature (Wen et al 2017). In both fens studied in detail in this work, the pH values were approximately neutral, potentially leading to a low abundance of *Methanoregula*. The acetoclastic methanogen, *Methanosaeta*, shows a consistent preference for neutral pH at the global and local scales, and is identified as a potential indicator lineage in pH neutral environments by our global biogeographic analysis. *Methanosaeta* is the most dominant methanogen in Zarnekow and Hütelmoor samples while other members from Methanosarcinaceae were underrepresented. Kemnitz et al (2004) demonstrated that Methanosaetaceae was only found in the permanently and frequently flooded soils with low concentrations of acetate (<30 μM). It is also confirmed by Galand et al (2005) that the low concentration of acetate in mesotrophic peats favors *Methanosaeta*, which have much higher affinity for low concentration of acetate than other acetoclastic methanogens like *Methanosarcina*. This is regarded as an evidence of niche adaption of methanogens under field conditions (Nazaries et al 2013). Moreover, according to our global analysis, acetoclastic methanogens are present in high frequencies in most environments (marine sediments, estuaries, lake sediments and soils), which emphasize their importance in global methane emissions,

as their contribution was estimated to be about 70% of total methane emission from natural environments (Conrad 1999).

At the local scale, aerobic methanotrophic bacteria exhibit distinct distribution in two rewetted peatlands, although methanotrophs account for a very low fraction among the whole bacterial population in both sites. In the coastal peatland Hütelmoor only type II (alphaproteobacterial) methanotrophs, mainly *Methylosinus* from the family Methylocystaceae were detected, whereas in Zarnekow only members of the genus *Crenothrix* and *Methylomonas* from type I (Gammaproteobacteria) methanotrophs were observed. The differences might be partially inherited from the different background (history) community compositions before rewetting. Moreover, type I methanotrophs are only detected in the very top layer (0-5cm) of Zarnekow samples, while the type II group spreads from 10 to 40 cm. The stratified differences may result from variations in oxygen and methane concentrations, as the surface layer is more dynamic and aerated. A soil microcosm study demonstrated that type I in contrast to type II methanotrophs respond more rapidly to different O_2/CH_4 ratios (Henckel et al 2000), therefore, type I may be adapted to the aerated microenvironment considering the transient nature of surface oxygenation. On the other hand, type II methanotrophs were suggested to be more stable in population structure, and mainly active under high methane mixing ratios (Henckel et al 2000). Further, the filamentous *Crenothrix* can thrive as major methane consumers under oxygen-rich and oxygen-deficient conditions on stratified lakes (Oswald et al 2017), which may have advantage to fit for conditions before and after drainage and restoration. However, the rewetted methanotrophic communities identified in this thesis present an incomplete picture of methanotrophic diversity in rewetted fens; therefore, an increasing sequencing effort with functional *pmoA* gene marker in more rewetted wetlands might enable us a more complete methanotrophic picture on a broader geographical scale.

5.3 Environmental selection versus dispersal limitation as potential factors shaping distribution patterns

A fundamental hypothesis for the biogeography of microorganisms is that 'everything is everywhere, but the environment selects' (Becking 1934, de Wit and Bouvier 2006). This hypothesis is based on the assumption that microorganisms have a cosmopolitan distribution. Due to their small sizes, microorganisms can easily and passively be dispersed everywhere, or in other words, that there is no dispersal limitation for microorganisms. The adaption and growth of microorganisms would be determined by the selective pressures in different environments or niches (environmental selection), where a given niche should support similar organisms regardless of geographic location (Fontaneto 2011). However, some studies also contradict the idea that 'everything is everywhere' (Martiny et al 2006) and imply that dispersal limitation contributes to the existence of bacterial or archaeal distribution patterns. To date, very few studies have attempted to address biogeographic patterns of methane-cycling microorganisms with respect to spatial scales and environmental parameters. In this thesis, a major focus of microbial biogeography was to identify whether the biogeographic pattern of methane-cycling microorganisms is primarily driven by environmental selection or by dispersal limitation.

The analysis on both global and local data reveals that methane-cycling microbial communities in natural environments and restored wetlands are more strongly regulated by environmental selection than by dispersal limitation. At global scale, a very weak correlation between methanogenic community structure and geographical distance is identified, whereas the environmental setting has a higher correlation to the community structure (Wen et al 2017). The methanogenic communities from soda lake sediments show more similar community structures with those from marine sediments which are geographically distant from each other, which is in contrast to larger differences between marine and freshwater environments. As transitional zone

between marine and freshwater habitats, methanogenic communities from estuaries cluster in between the marine and freshwater communities in the ordination space. The statistical analysis on methanogenic communities in European soils and lake sediments support the trend that the community structure is more associated with environmental conditions than with geographical distance, as some geographically very close sites show dissimilar methanogenic community composition, while some geographically distant sites have very similar communities.

The importance of environmental selection on the methanogenic community is also demonstrated by the local data (Wen et al 2018). In both rewetted fens, the geochemical heterogeneity is reflected in methanogenic and methanotrophic community structure, regardless of geographical distance. For example, Hütelmoor core (HC) 1, whose geochemical condition is different to other Hütelmoor cores and more similar to the Zarnekow cores, displays similar methanogenic community structures to the Zarnekow samples, even though it is geographically more close to other Hütelmoor cores. This trend occurs not only for methanogens, but is also reflected in the whole archaeal and bacterial communities. Another important aspect is that the two restored environments, Hütelmoor and Zarnekow, have a similar low ratio of methanotrophic to methanogenic abundances. Relative abundances of aerobic methanotrophs are very low in both sites. This indicates the environmental disturbances caused by rewetting, e.g., in our case by flooding, have an overall strong impact on methanotrophic communities and their re-establishment.

The strong influence of environmental selection over dispersal limitation on methane-cycling microorganisms at different spatial scales implies that, methane-cycling microorganisms are not randomly distributed over space. It is the selective pressure in different environments determines the subsequent growth of these organisms. If their physiological requirements are met by local environment conditions they could be able to quickly establish stable communities.

5.4 Environmental drivers of methane-cycling microbial communities

With the aim of improving our knowledge on the distribution and environmental controls of the microbial communities of the methane cycle as well as their responses to climate and environmental change in key habitats, the correlations between microbial community composition and environmental conditions at different spatial scales are explored. According to IPCC fifth's assessment report, climate changes are predicted to be expressed in rising temperature, changes in the amount, intensity, and seasonal distribution of precipitation and amount of snow fall and cover, and increase frequency of flooding events (Pachauri et al 2014). In this thesis, I focused on salinity, pH and temperature as the global biogeography study identified them as main environmental drivers of methanogenic community.

At the global scale, salinity is identified as important driver which substantially explains the methanogenic community distribution patterns in multiple habitats (Wen et al 2017). Salinity reflects the amounts of various inorganic minerals or salts dissolved in a given volume of water, which can define the osmotic pressure, available electron donor or acceptors. Salinity is required to maintain osmotic pressure. Increase in salinity (hyperosmotic stress) causes the water to move out of the cell, leading to a loss of cell turgor pressure, change of the cell volume, and variations in local ion distributions (Daniel et al 2004). In a related global study, salinity was recognized as a key factor in regulating archaeal community structure in both terrestrial and aquatic habitats (Auguet et al 2010). A very recent study showed that community salt tolerance was closely correlated with soil salinity, indicating a strong filtering effect of salinity on the bacterial communities (Rath et al 2019). The importance of salinity is also highlighted by our local analysis. Methane-cycling microbial communities in two rewetted fens, a coastal brackish fen (Hütelmoor) and a freshwater riparian fen (Zarnekow), showed salinity as a significant driving force in shaping both bacterial and archaeal community composition. I further compared the

optimum concentration of NaCl for growth of methanogenic pure cultures, which shows that isolates from marine and hydrothermal sediments have significantly higher requirement for NaCl than those from soils. As such, the prevalence of these taxa may serve as potential bioindicators for high salt tolerance communities (Rath et al 2019). Salinity, even under different selective pressures, still emerged as a primary dominant factor linked to microbial community composition (Lozupone and Knight 2007). These findings highlight the importance of salinity in regulating microbial distribution at global scale.

The salinity of a given environment is dynamic and changes with environmental factors, for example with rainfall, temperature and plant cover (Várallyay 1994). Globally, salinization is occurring at an unprecedented rate and geographic scale, representing a threat to the structure and ecological functioning of inland and coastal wetlands (Herbert et al 2015, van Dijk et al 2015). In addition, global warming increases the salinity of the sea, owing to the increased evaporation and reduced rainfall for example across a giant stretch of water from Africa to the Caribbean in recent years (Stott et al 2008). Theoretically, increased salinity can stimulate microbial mineralization of organic matter on the long run due to introducing more terminal electron acceptors such as Fe^{3+}, Mn^{4+}, SO_4^{2-}, potentially leading to shifts in the dominant pathway of anaerobic metabolism from methanogenesis towards higher energy-yielding pathways (e.g., SO_4^{2-} reduction) (Herbert et al 2015, van Dijk et al 2015). Elevated salinity has been found to reduce both aerobic (van der Gon and Neue 1995) and anaerobic methanotrophy, with the aerobic microorganisms being especially sensitive to salinity (Dalal et al 2008). Salinity changes in coastal lowlands, coastal soils and wetlands can be associated with sea level rise, land subsidence and altered hydrological and climatic conditions (van Dijk et al 2015, Várallyay 1994). As important habitat of methane-cycling microorganisms, estuaries, intertidal zones and coastal wetlands may face salinity changes under global warming that would further alter the microbial community structure of methane cycle.

In this thesis, pH is identified as an important environmental variable that regulates the methanogenic community in globally distributed nonsaline soil and lake sediment samples, while at local scale pH is found to be one of the key factors influencing microbial community composition in the two rewetted fens. A similar result has been frequently identified in various habitats such as soils (Fierer and Jackson 2006, Jones et al 2009, Nicol et al 2008, Ren et al 2018, Rousk et al 2010), farmland or land use change (Bartram et al 2014, Liu et al 2018), contaminated environment (Wu et al 2017), oral (Bowden and Hamilton 1987) and human gut microbiome (Sofi et al 2014). Methanogens were found to be very sensitive to variable pH (Wang et al 1993). A possible reason is that hydrogenotrophic methanogens are generally more acid-tolerant than acetoclastic methanogens (Horn et al 2003, Kotsyurbenko et al 2007). Conversely, methanotrophs seem to be more tolerant to pH variations than methanogens. Kolb (2009) implemented a meta-analysis of methanotrophic communities in 53 globally distributed soils and indicated that the type II (alphaproteobacterial) methanotrophs and members of USCα were dominant in acidic soils, while type I (gammaproteobacterial) and members of USCγ methanotrophs were frequently detected in pH-neutral soils.

Soil pH can be an important variable affecting nutrient availability by controlling the chemical forms of the different nutrients and influencing the chemical reactions. Changes in pH due to land use were able to affect microbial carbon cycling processes through controlling microbial mechanisms of carbon accumulation, as suggested by a meta-study on 56 geographically distributed sites across UK (Malik et al 2018). Several studies have suggested a linkage between increasing pH and increase of the proportion of CH_4 produced by acetate cleavage relative to CO_2 reduction (Hines et al 2008, Kotsyurbenko et al 2007). Another study showed that increasing organic matter lability and pH induced by permafrost thawing is consistent with a shift from hydrogenotrophic to acetoclastic methanogenesis (Hodgkins et al 2014). In the context of climate warming, the soil pH change in natural environments may be also associated with modification of plant and microbial activity. For example, a study revealed associations between elevated soil pH with succession of plant community

along a permafrost thaw sequence, which consequently influenced the dissolved organic carbon and greenhouse gas emission (Hodgkins et al 2014). However, such pH change might take place and accumulate on the long run. Agricultural amendment of fertilizer may modify pH more rapidly and considerably (Barak et al 1997). Especially in wetland that has been converted for agricultural use and was recently rewetted, the change of pH may exert rapid impact during the reconstruction of microbial community and their activities.

Temperature is another important environmental variable identified here to regulate methanogenic community composition in nonsaline soils and lake sediments at the global scale. In the two rewetted fens, temperature was not taken into account because of negligible temperature difference on the two individual local sites. Temperature change has a positive effect on methanogenesis but has slight effect on methanotrophy (Nazaries et al 2013). The direct effects of temperature on microbial physiology are thought to be mediated by microbial adaptations, evolution, and interactions over time, and indirect effects are due to changes in soil moisture which is often coupled with temperature changes (Classen et al 2015). Global warming can directly alter microbial soil respiration rates because soil microorganisms, and the processes they mediate, are temperature sensitive (Classen et al 2015). Warming by 5°C in a temperate forest, for example, was able to alter the relative abundances of soil bacteria and increase the bacterial to fungal ratio of the community (DeAngelis et al 2015). Tveit et al (2015) conducted a temperature-gradient study showing that the methane production rates depended on temperature following the Ratkowsky equation (square root model) within the temperature range (1-30 °C) studied. However, temperature will induce compositional and functional shifts at different critical temperature windows at which adaptive reconstruction of microbial consortia can overcome metabolic bottlenecks of anaerobic carbon degradation pathways (Tveit et al 2015). Temperature rising receives special concerns in polar regions due to its massive carbon content and fatser warming rate (IPCC 2007). The consequent permafrost thawing will accelerate greenhouse gas emission through direct enhancement on cell growth and metabolism, and indirect influence on the

geochemical conditions around microorganisms (Farrell and Rose 1967, Schuur et al 2011). The deepening of active layer is a very common consequence of permafrost warming (Huggett 2016, Romanovsky and Osterkamp 1997). Additionally, the deepening of the active layer can expand the spatial extent for viable microbial community. If recent trends of warming continue, the consequent release of greenhouse gases from thawed permafrost to the atmosphere represent a positive feedback to the global warming.

5.5 Conclusion

This thesis is the first to present a global biogeographic map and analysis of methanogenic archaea in various natural environments (summarized in Figure 5.1). Non-marine and transitional habitats (soils, lake sediments and estuaries) harbor higher methanogenic diversity than marine habitats (marine sediments, hydrothermal sediments and mud volcanos). Even though some lineages were observed in multiple habitats, methanogens in general show habitat preferences. Accounting for the local and regional scale, this work focused on the distribution of methane-cycling microorganisms in two rewetted fens in northeastern Germany. According to different geochemical conditions, the two rewetted fens differ in overall bacterial and archaeal community structure. However, similar low ratios of methanotrophic to methanogenic abundances identified in both fens indicate the successful re-establishment of methanogens and a slow re-establishment of methanotrophs after rewetting.

Combining the global and local analysis, the study has demonstrated that the distribution patterns of methane-cycling microbial community in natural environments and restored wetlands are more associated with habitat filtering than with dispersal limitation. The microbial community structure of methanogens and methanotrophs is regulated by environmental factors, such as salinity, pH and temperature (summarized in Figure 5.1). With these environmental parameters, the response of methane-cycling microorganisms to potentially drastic environmental

changes should be taken into account in predicting the feedback of methane emissions to future climate change.

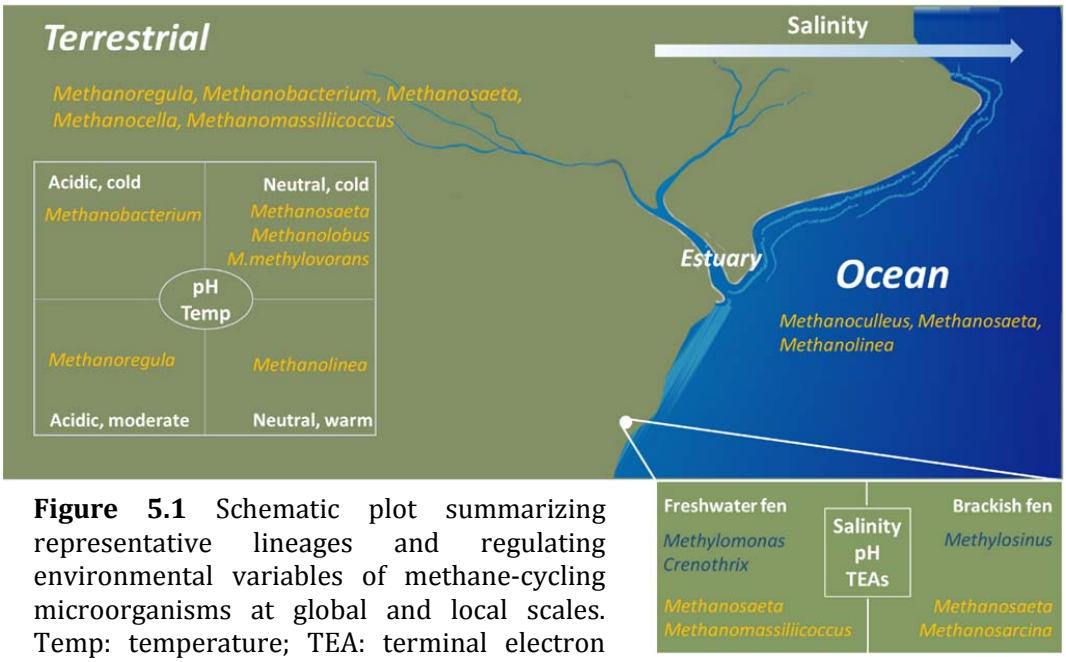


Figure 5.1 Schematic plot summarizing representative lineages and regulating environmental variables of methane-cycling microorganisms at global and local scales. Temp: temperature; TEA: terminal electron acceptor.

5.6 Outlook

In this thesis, the globally distributed public datasets were collected and analyzed to determine the distribution patterns and environmental drivers of methanogenic communities. Despite of the mounting sequencing data in the public database, a limitation for identifying the drivers of the distribution patterns of methanogens is the lack of consistent abiotic and biotic factors (metadata) provided by different studies. The common parameters which were available for analysis could only explain a partial variation of the methanogenic community composition. The local study of two rewetted fens identified oxygen and available alternative terminal electron acceptors as important factors influencing microbial community structure, but these two environmental variables are not available in most of the published studies.

Furthermore, it is possible that the relative importance of driving environmental factors of specific microorganisms differ at different scales. Therefore, more available metadata will help us to get a more comprehensive interpretation of the environmental variables which shape these important microorganisms at different scales in the future. It would be a great step forward for future meta-studies if the environmental microbiology society came up with a standard of minimum environmental variables for sequence data like what was recently advocated by Glass et al (2014). In recent years, NGS techniques have been extensively used to study multiple sites at local or even regional scales, which have yields a massive amount of data with fine-resolution molecular signatures in a single run. Integrating these meta-data into biogeography studies, or direct experiment targeted at large scales will improve and refine our knowledge of microbial biogeography patterns and the regulating mechanisms.

Moreover, recent studies have shown that there is substantial *mcrA* diversity outside of the Euryarchaeota phylum. For example, some members of Bathyarchaeota, a phylum that is widespread in anoxic sediments, were found containing genes necessary for methane metabolism, and were suggested as possible methylotrophic methanogens (Evans et al. 2015). This indicates that the diversity of methanogens may be larger than previously expected. Therefore, further attempts to conduct meta-analysis by using NGS data and including primers for *mcrA* in general, is necessary to explore potential methanogenic taxa that still remain to be verified as methanogens.

Rewetting, or sometimes simply flooding of drained wetlands, as the most prevalent restoration method, aims to re-establish the conditions of pristine wetland by raising water table to its initial state (Evans et al 2005, Holden et al 2011). Because of the increased water table and water saturation in soils, rewetting can lead to increasing methane emission (Saarnio et al 2009). In our two studied rewetted fens, high methane emissions were observed and these high emissions even last for decades following flooding. Our microbial data suggests that the rapid re-establishment of methanogenic community and slow re-establishment of methanotrophic community

together contribute to prolonged increased methane emissions. Therefore, long-term monitoring and investigation of the dynamics of microbial communities in natural and degraded wetlands as well as their re-establishment after rewetting are crucial for assessing the suitability and the management of wetland restoration processes. During rewetting process, the anaerobic methane oxidizers, as suggested by ANMEs in the rewetted samples, may partially take over the oxidation of methane and thus their roles, dynamics should be taken into account to get comprehensive understanding about methane-associated consortia. Furthermore, ecological data from DNA markers based studies could not highlight the active members of a given methanogenic and methanotrophic community in rewetted wetlands. In this case, combining the RNA-based approaches such as transcriptomics or metatranscriptomics will enable us to profile both community composition and functional establishment of methane-cycling microorganisms in these environments.

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. *Ecol Evol* **6:** 7080-7102.

Abril G, Iversen N (2002). Methane dynamics in a shallow non-tidal estuary (Randers Fjord, Denmark). *Mar Ecol Prog Ser* **230:** 171-181.

Abril G, Borges AV (2005). Carbon Dioxide and Methane Emissions from Estuaries. In: Tremblay A, Varfalvy L, Roehm C, Garneau M (eds). *Greenhouse Gas Emissions - Fluxes and Processes: Hydroelectric Reservoirs and Natural Environments*. Springer Berlin Heidelberg: Berlin, Heidelberg. pp 187-207.

Adam N, Perner M (2018). Microbially mediated hydrogen cycling in deep-sea hydrothermal vents. *Front Microbiol* **9:** 2873.

Agethen S, Knorr K-H (2018). 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 Biol Biochem* **117:** 139-152.

Aharon O (2014). The Family Methanomicrobiaceae. In: Rosenberg E., et al. (eds.), *The Prokaryotes – Other Major Lineages of Bacteria and the Archaea*. Springer-Verlag Berlin Heidelberg. pp 234-237.

Angel R, Claus P, Conrad R (2012). Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. *ISME J* **6:** 847-862.

Angelis MA, Scranton MI (1993). Fate of methane in the Hudson River and estuary. *Global Biogeochem Cy* **7:** 509-523.

Antony CP, Murrell JC, Shouche YS (2012). Molecular diversity of methanogens and identification of Methanolobus sp. as active methylotrophic Archaea in Lonar crater lake sediments. *FEMS Microbiol Ecol* **81:** 43-51.

Auguet JC, Barberan A, Casamayor EO (2010). Global ecological patterns in uncultured Archaea. *ISME J* **4:** 182-190.

Augustin J, Merbach W, Rogasik J (1998). Factors influencing nitrous oxide and methane emissions from minerotrophic fens in northeast Germany. *Biol Fertil Soils* **28:** 1-4.

Augustin J, Joosten H (2007). Peatland rewetting and the greenhouse effect. *International Mire Conservation Group Newsletter* **3:** 29-30.

Augustin J, Chojnicki B (2008). Austausch von klimarelevanten Spurengasen, Klimawirkung und Kohlenstoffdynamik in den ersten Jahren nach der Wiedervernässung von degradiertem

Niedermoorgrünland. *Berichte des Leibniz-Institut für Gewässerökologie und Binnenfischerei* **26:** 47-67.

Banning N, Brock F, Fry JC, Parkes RJ, Hornibrook ER, Weightman AJ (2005). Investigation of the methanogen population structure and activity in a brackish lake sediment. *Environ Microbiol* **7:** 947-960.

Barak P, Jobe BO, Krueger AR, Peterson LA, Laird DA (1997). Effects of long-term soil acidification due to nitrogen fertilizer inputs in Wisconsin. *Plant Soil* **197:** 61-69.

Barbier BA, Dziduch I, Liebner S, Ganzert L, Lantuit H, Pollard W *et al* (2012). Methane-cycling communities in a permafrost-affected soil on Herschel Island, Western Canadian Arctic: active layer profiling of *mcrA* and *pmoA* genes. *FEMS Microbiol Ecol* **82:** 287-302.

Barreto DP, Conrad R, Klose M, Claus P, Enrich-Prast A (2014). Distance-decay and taxa-area relationships for bacteria, archaea and methanogenic archaea in a tropical lake sediment. *Plos One* **9:** e110128.

Bartlett KB, Bartlett DS, Harriss RC, Sebacher DI (1987). Methane emissions along a salt marsh salinity gradient. *Biogeochemistry* **4:** 183-202.

Bartram AK, Jiang X, Lynch MDJ, Masella AP, Nicol GW, Dushoff J *et al* (2014). Exploring links between pH and bacterial community composition in soils from the Craibstone Experimental Farm. *FEMS Microbiol Ecol* **87:** 403-415.

Bastviken D, Cole J, Pace M, Tranvik L (2004). Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. *Global Biogeochem Cy* **18:** GB4009.

Becking LB (1934). *Geobiologie of inleiding tot de milieukunde*. WP Van Stockum & Zoon.

Beulig F, Heuer VB, Akob DM, Viehweger B, Elvert M, Herrmann M *et al* (2015). Carbon flow from volcanic CO_2 into soil microbial communities of a wetland mofette. *ISME J* **9:** 746-759.

Biddle JF, Cardman Z, Mendlovitz H, Albert DB, Lloyd KG, Boetius A *et al* (2012). Anaerobic oxidation of methane at different temperature regimes in Guaymas Basin hydrothermal sediments. *ISME J* **6:** 1018-1031.

Biderre-Petit C, Jezequel D, Dugat-Bony E, Lopes F, Kuever J, Borrel G *et al* (2011). Identification of microbial communities involved in the methane cycle of a freshwater meromictic lake. *FEMS Microbiol Ecol* **77:** 533-545.

Birdsell JA (2002). Integrating genomics, bioinformatics, and classical genetics to study the effects of recombination on genome evolution. *Mol Biol Evol* **19:** 1181-1197.

Bodelier PLE, Bar-Gilissen MJ, Meima-Franke M, Hordijk K (2012). Structural and functional response of methane-consuming microbial communities to different flooding regimes in riparian soils. *Ecol Evol* **2:** 106-127.

Bolger AM, Lohse M, Usadel B (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30:** 2114-2120.

Borrel G, O'Toole PW, Harris HM, Peyret P, Brugere J-F, Gribaldo S (2013). Phylogenomic data support a seventh order of methylotrophic methanogens and provide insights into the evolution of methanogenesis. *Genome Biol Evol* **5:** 1769-1780.

Bousquet P, Ciais P, Miller JB, Dlugokencky EJ, Hauglustaine DA, Prigent C *et al* (2006). Contribution of anthropogenic and natural sources to atmospheric methane variability. *Nature* **443:** 439-443.

Bowden GH, Hamilton IR (1987). Environmental pH as a factor in the competition between strains of the oral streptococci *Streptococcus mutans*, *S. sanguis*, and "*S. mitior*" growing in continuous culture. *Can J Microbiol* **33:** 824-827.

Bowman JP, McCammon SA, Skerrat JH (1997). Methylosphaera hansonii gen. nov., sp. nov., a psychrophilic, group I methanotroph from Antarctic marine-salinity, meromictic lakes. *Microbiology* **143:** 1451-1459.

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

Burgos M, Sierra A, Ortega T, Forja J (2015). Anthropogenic effects on greenhouse gas (CH_4 and N_2O) emissions in the Guadalete River Estuary (SW Spain). *Sci Total Environ* **503:** 179-189.

Buriánková I, Brablcová L, Mach V, Dvořák P, Chaudhary PP, Rulík M (2013). Identification of Methanogenic archaea in the hyporheic sediment of Sitka stream. *Plos One* **8:** e80804.

Bussmann I (2013). Distribution of methane in the Lena Delta and Buor Khaya Bay, Russia. *Biogeosciences* **10:** 4641-4652.

Cadillo-Quiroz H, Yashiro E, Yavitt JB, Zinder SH (2008). Characterization of the archaeal community in a minerotrophic fen and terminal restriction fragment length polymorphism-directed isolation of a novel hydrogenotrophic methanogen. *Appl Environ Microbiol* **74:** 2059-2068.

Cao H, Auguet J-C, Gu J-D (2013). Global ecological pattern of ammonia-oxidizing archaea. *Plos One* **8:** e52853.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK *et al* (2010). QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7:** 335.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ *et al* (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci USA* **108:** 4516-4522.

Castro H, Ogram A, Reddy KR (2004). Phylogenetic characterization of methanogenic assemblages in eutrophic and oligotrophic areas of the Florida Everglades. *Appl Environ Microbiol* **70:** 6559-6568.

Chaffron S, Rehrauer H, Pernthaler J, von Mering C (2010). A global network of coexisting microbes from environmental and whole-genome sequence data. *Genome Res* **20:** 947-959.

Chambers LG, Guevara R, Boyer JN, Troxler TG, Davis SE (2016). Effects of salinity and inundation on microbial community structure and function in a mangrove peat soil. *Wetlands* **36:** 361-371.

Chasar LS, Chanton JP, Glaser PH, Siegel DI (2000). 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* **86:** 655-663.

Cheema S, Zeyer J, Henneberger R (2015). Methanotrophic and methanogenic communities in Swiss alpine fens dominated by *Carex rostrata* and *Eriophorum angustifolium*. *Appl Environ Microbiol* **81:** 5832-5844.

Chen Y, Dumont MG, Cébron A, Murrell JC (2007). Identification of active methanotrophs in a landfill cover soil through detection of expression of 16S rRNA and functional genes. *Environ Microbiol* **9:** 2855-2869.

Chessel D, Dufour AB, Thioulouse J (2004). The ade4 package-I: One-table methods. *R news* **4:** 5-10.

Christiansen JR, Romero AJB, Jørgensen NOG, Glaring MA, Jørgensen CJ, Berg LK *et al* (2014). Methane fluxes and the functional groups of methanotrophs and methanogens in a young Arctic landscape on Disko Island, West Greenland. *Biogeochemistry* **122:** 15-33.

Church MJ, Björkman KM, Karl DM, Saito MA, Zehr JP (2008). Regional distributions of nitrogen-fixing bacteria in the Pacific Ocean. *Limnol Oceanogr* **53:** 63-77.

Classen AT, Sundqvist MK, Henning JA, Newman GS, Moore JA, Cregger MA *et al* (2015). Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? *Ecosphere* **6:** 1-21.

Conrad R (1996). Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, CO_2, N_2O, and NO). *Microbiol Rev* **60:** 609-640.

Conrad R (1999). Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. *FEMS Microbiol Ecol* **28:** 193-202.

Conrad R (2007). Microb Ecol of methanogens and methanotrophs. *Adv Agron* **96:** 1-63.

Conrad R, Klose M, Claus P, Enrich-Prast A (2010). Methanogenic pathway, ^{13}C isotope fractionation, and archaeal community composition in the sediment of two clear-water lakes of Amazonia. *Limnol Oceanogr* **55:** 689-702.

Conrad R, Ji Y, Noll M, Klose M, Claus P, Enrich-Prast A (2014). Response of the methanogenic microbial communities in Amazonian oxbow lake sediments to desiccation stress. *Environ Microbiol* **16:** 1682-1694.

Cooper MD, Evans CD, Zielinski P, Levy PE, Gray A, Peacock M *et al* (2014). Infilled ditches are hotspots of landscape methane flux following peatland re-wetting. *Ecosystems* **17:** 1227-1241.

Cornwell WK, Schwilk DW, Ackerly DD (2006). A trait-based test for habitat filtering: convex hull volume. *Ecology* **87:** 1465-1471.

Costello AM, Lidstrom ME (1999). Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. *Appl Environ Microbiol* **65:** 5066-5074.

Costello AM, Auman AJ, Macalady JL, Scow KM, Lidstrom ME (2002). Estimation of methanotroph abundance in a freshwater lake sediment. *Environ Microbiol* **4:** 443-450.

Crump BC, Amaral-Zettler LA, Kling GW (2012). Microbial diversity in arctic freshwaters is structured by inoculation of microbes from soils. *ISME J* **6:** 1629-1639.

Cui M, Ma A, Qi H, Zhuang X, Zhuang G, Zhao G (2015). Warmer temperature accelerates methane emissions from the Zoige wetland on the Tibetan Plateau without changing methanogenic community composition. *Sci Rep* **5:** 11616.

Cunliffe M, Schäfer H, Harrison E, Cleave S, Upstill-Goddard R, Murrell JC (2008). Phylogenetic and functional gene analysis of the bacterial and archaeal communities associated with the surface microlayer of an estuary. *ISME J* **2:** 776–789.

Dalal RC, Allen DE, Livesley SJ, Richards G (2008). Magnitude and biophysical regulators of methane emission and consumption in the Australian agricultural, forest, and submerged landscapes: a review. *Plant Soil* **309:** 43-76.

Daniel RM, Finney JL, Stoneham M, Grant WD (2004). Life at low water activity. *Philos Trans R Soc Lond B Biol Sci* **359:** 1249-1267.

de Wit R, Bouvier T (2006). 'Everything is everywhere, but, the environment selects'; what did Baas Becking and Beijerinck really say? *Environ Microbiol* **8:** 755-758.

Dean JF, J. MJ, Thomas R, Rien A, G. BL, Matthias E *et al* (2018). Methane feedbacks to the global climate system in a warmer world. *Rev* Geophys **56:** 207-250.

DeAngelis KM, Pold G, Topçuoğlu BD, van Diepen LTA, Varney RM, Blanchard JL *et al* (2015). Long-term forest soil warming alters microbial communities in temperate forest soils. *Front Microbiol* **6:** doi:10.3389/fmicb.2015.00104.

Dedysh SN, Liesack W, Khmelenina VN, Suzina NE, Trotsenko YA, Semrau JD *et al* (2000). Methylocella palustris gen. nov., sp. nov., a new methane-oxidizing acidophilic bacterium from peat bogs, representing a novel subtype of serine-pathway methanotrophs. *Int J Syst Evol Microbiol* **50:** 955-969.

Degelmann DM, Borken W, Drake HL, Kolb S (2010). Different atmospheric methane-oxidizing communities in European beech and Norway spruce soils. *Appl Environ Microbiol* **76:** 3228-3235.

Dhillon A, Lever M, Lloyd KG, Albert DB, Sogin ML, Teske A (2005). Methanogen diversity evidenced by molecular characterization of methyl coenzyme M reductase A (*mcrA*) genes in hydrothermal sediments of the Guaymas Basin. *Appl Environ Microbiol* **71:** 4592-4601.

Dray S, Dufour A-B (2007). The ade4 package: implementing the duality diagram for ecologists. *J Stat Softw* **22:** 1-20.

Dufrêne M, Legendre P (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol Monograph* **67:** 345-366.

Dumont MG, Murrell JC (2005). Community-level analysis: key genes of aerobic methane oxidation. *Methods Enzymol* **397:** 413-427.

Dumont MG, Pommerenke B, Casper P, Conrad R (2011). DNA-, rRNA- and mRNA-based stable isotope probing of aerobic methanotrophs in lake sediment. *Environ Microbiol* **13:** 1153-1167.

Dumont MG, Luke C, Deng YC, Frenzel P (2014). Classification of *pmoA* amplicon pyrosequences using BLAST and the lowest common ancestor method in MEGAN. *Front Microbiol* **5:** 34. doi: 10.3389/fmicb. 2014.00034.

Dunfield PF, Yuryev A, Senin P, Smirnova AV, Stott MB, Hou S *et al* (2007). Methane oxidation by an extremely acidophilic bacterium of the phylum *Verrucomicrobia*. *Nature* **450:** 879-882.

Earl J, Hall G, Pickup R, Ritchie D, Edwards C (2003). Analysis of methanogen diversity in a hypereutrophic lake using PCR-RFLP analysis of mcr sequences. *Microb Ecol* **46:** 270-278.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27:** 2194-2200.

Eller G, Kruger M, Frenzel P (2005). Comparing field and microcosm experiments: a case study on methano- and methylo-trophic bacteria in paddy soil. *FEMS Microbiol Ecol* **51:** 279-291.

Emsens W-J, Aggenbach CJS, Schoutens K, Smolders AJP, Zak D, van Diggelen R (2016). Soil Iron content as a predictor of carbon and nutrient mobilization in rewetted fens. *Plos One* **11:** e0153166.

Erkel C, Kube M, Reinhardt R, Liesack W (2006). Genome of Rice Cluster I archaea - the key methane producers in the rice rhizosphere. *Science* **313:** 370-372.

Ettwig KF, Zhu B, Speth D, Keltjens JT, Jetten MSM, Kartal B (2016). Archaea catalyze iron-dependent anaerobic oxidation of methane. *Proc Natl Acad Sci USA* **113:** 12792-12796.

Evans CD, Monteith DT, Cooper DM (2005). Long-term increases in surface water dissolved organic carbon: Observations, possible causes and environmental impacts. *Environ Pollut* **137:** 55-71.

Evans PN, Parks DH, Chadwick GL, Robbins SJ, Orphan VJ, Golding SD *et al* (2015). Methane metabolism in the archaeal phylum *Bathyarchaeota* revealed by genome-centric metagenomics. *Science* **350:** 434-438.

Evans PN, Boyd JA, Leu AO, Woodcroft BJ, Parks DH, Hugenholtz P *et al* (2019). An evolving view of methane metabolism in the Archaea. *Nat Rev Microbiol* **17:** 219-232.

Farrell J, Rose A (1967). Temperature effects on microorganisms. *Ann Rev Microbiol* **21:** 101-120.

Fierer N, Jackson RB (2006). The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci USA* **103:** 626-631.

Fish JA, Chai B, Wang Q, Sun Y, Brown CT, Tiedje JM *et al* (2013). FunGene: the functional gene pipeline and repository. *Front Microbiol* **4:** 291.

Fontaneto D (2011). *Biogeography of microscopic organisms: is everything small everywhere?* Cambridge University Press: Cambridge.

Francez A-J, Gogo S, Josselin N (2000). Distribution of potential CO_2 and CH_4 productions, denitrification and microbial biomass C and N in the profileof a restored peatland in Brittany (France). *Eur J Soil Biol* **36:** 161-168.

Franchini AG, Zeyer J (2012). Freeze-coring method for characterization of microbial community structure and function in wetland soils at high spatial resolution. *Appl Environ Microbiol* **78:** 4501-4504.

Franchini AG, Henneberger R, Aeppli M, Zeyer J (2015). Methane dynamics in an alpine fen: a field-based study on methanogenic and methanotrophic microbial communities. *FEMS Microbiol Ecol* **91:** doi:10.1093/FEMSec/fiu1032.

Frank-Fahle BA, Yergeau E, Greer CW, Lantuit H, Wagner D (2014). Microbial functional potential and community composition in permafrost-affected soils of the NW Canadian Arctic. *Plos One* **9:** e84761.

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.

Freitag TE, Prosser JI (2009). Correlation of methane production and functional gene transcriptional activity in a peat soil. *Appl Environ Microbiol* **75:** 6679-6687.

Freitag TE, Toet S, Ineson P, Prosser JI (2010). Links between methane flux and transcriptional activities of methanogens and methane oxidizers in a blanket peat bog. *FEMS Microbiol Ecol* **73:** 157-165.

Frolking S, Talbot J, Jones MC, Treat CC, Kauffman JB, Tuittila E-S *et al* (2011). Peatlands in the Earth's 21st century climate system. *Environ Rev* **19:** 371-396.

Fukuzaki S, Nishio N, Nagai S (1990). Kinetics of the methanogenic fermentation of acetate. *Appl Environ Microbiol* **56:** 3158-3163.

Galand PE, Saarnio S, Fritze H, Yrjälä K (2002). Depth related diversity of methanogen Archaea in Finnish oligotrophic fen. *FEMS Microbiol Ecol* **42:** 441-449.

Galand PE, Fritze H, Conrad R, Yrjala K (2005). Pathways for methanogenesis and diversity of methanogenic archaea in three boreal peatland ecosystems. *Appl Environ Microbiol* **71:** 2195-2198.

Garrity GM, Labeda DP, Oren A (2011). Judicial Commission of the International Committee on Systematics of Prokaryotes XIIth International (IUMS) Congress of Bacteriology and Applied Microbiology. *Int J Syst Evol Microbiol* **61:** 2775-2780.

Glass EM, Dribinsky Y, Yilmaz P, Levin H, Van Pelt R, Wendel D et al (2014). MIxS-BE: a MIxS extension defining a minimum information standard for sequence data from the built environment. *ISME J* **8**:1-3.

Graef C, Hestnes AG, Svenning MM, Frenzel P (2011). The active methanotrophic community in a wetland from the High Arctic. *Environ Microbiol Rep* **3:** 466-472.

Großkopf R, Janssen PH, Liesack W (1998). Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. *Appl Environ Microbiol* **64:** 960-969.

Grunwald M, Dellwig O, Beck M, Dippner JW, Freund JA, Kohlmeier C *et al* (2009). Methane in the southern North Sea: Sources, spatial distribution and budgets. *Estuar Coast Shelf Sci* **81:** 445-456.

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 CH_4 and CO_2. *Biogeosciences* **8:** 1539-1550.

Hahn J, Kohler S, Glatzel S, Jurasinski G (2015). Methane exchange in a coastal fen in the first year after flooding - a systems shift. *Plos One* **10:** e0140657.

Hakemian AS, Rosenzweig AC (2007). The biochemistry of methane oxidation. *Annu Rev Biochem* **76:** 223-241.

Hallam SJ, DeLong EF, Ussler III W, Paull CK, Winters W, Lorenson T *et al* (2007). Methanogenic and Methanotrophic Archaeal Diversity in Northern Gulf of Mexico Sediments. In: Winters W, Loren-son T, Paull C (eds). *Initial report of the IMAGES VIII/ gas hydrate and paleoclimate cruise on the RV Marion Dufresne in the Gulf of Mexico*. Chapter 10.

Hanson CA, Fuhrman JA, Horner-Devine MC, Martiny JB (2012). Beyond biogeographic patterns: processes shaping the microbial landscape. *Nat Rev Microbiol* **10:** 497-506.

Hanson RS, Hanson TE (1996). Methanotrophic bacteria. *Microbiol Rev* **60:** 439-471.

Haroon MF, Hu S, Shi Y, Imelfort M, Keller J, Hugenholtz P *et al* (2013). Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. *Nature* **500:** 567.

Harrison BK, Zhang H, Berelson W, Orphan VJ (2009). Variations in archaeal and bacterial diversity associated with the sulfate-methane transition zone in continental margin sediments (Santa Barbara Basin, California). *Appl Environ Microbiol* **75:** 1487-1499.

He S, Malfatti SA, McFarland JW, Anderson FE, Pati A, Huntemann M *et al* (2015). Patterns in wetland microbial community composition and functional gene repertoire associated with methane emissions. *mBio* **6:** e00066-00015.

Henckel T, Roslev P, Conrad R (2000). Effects of O_2 and CH_4 on presence and activity of the indigenous methanotrophic community in rice field soil. *Environ Microbiol* **2:** 666-679.

Herbert ER, Boon P, Burgin AJ, Neubauer SC, Franklin RB, Ardón M *et al* (2015). A global perspective on wetland salinization: ecological consequences of a growing threat to freshwater wetlands. *Ecosphere* **6:** doi:10.1890/es1814-00534.00531.

Herlemann DP, Labrenz M, Jürgens K, Bertilsson S, Waniek JJ, Andersson AF (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J* **5:** 1571-1579.

Hernandez ME, Beck DAC, Lidstrom ME, Chistoserdova L (2015). Oxygen availability is a major factor in determining the composition of microbial communities involved in methane oxidation. *PeerJ* **3:** e801.

Heyer J, Galchenko VF, Dunfield PF (2002). Molecular phylogeny of type II methane-oxidizing bacteria isolated from various environments. *Microbiology* **148:** 2831-2846.

Hines ME, Duddleston KN, Rooney-Varga JN, Fields D, Chanton JP (2008). Uncoupling of acetate degradation from methane formation in Alaskan wetlands: Connections to vegetation distribution. *Global Biogeochem Cy* **22:** doi:10.1029/2006GB002903.

Ho A, Luke C, Frenzel P (2011). Recovery of methanotrophs from disturbance: population dynamics, evenness and functioning. *ISME J* **5:** 750-758.

Ho A, Frenzel P (2012). Heat stress and methane-oxidizing bacteria: Effects on activity and population dynamics. *Soil Biol Biochem* **50:** 22-25.

Ho A, Kerckhof F-M, Luke C, Reim A, Krause S, Boon N *et al* (2013). Conceptualizing functional traits and ecological characteristics of methane-oxidizing bacteria as life strategies. *Environ Microbiol Rep* **5:** 335-345.

Hodgkins SB, Tfaily MM, McCalley CK, Logan TA, Crill PM, Saleska SR *et al* (2014). Changes in peat chemistry associated with permafrost thaw increase greenhouse gas production. *Proc Natl Acad Sci USA* **111:** 5819-5824.

Holden J, Wallage ZE, Lane SN, McDonald AT (2011). Water table dynamics in undisturbed, drained and restored blanket peat. *J Hydrol* **402:** 103-114.

Holm GO, Perez BC, McWhorter DE, Krauss KW, Johnson DJ, Raynie RC *et al* (2016). 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.

Holmes AJ, Costello A, Lidstrom ME, Murrell JC (1995). Evidence that particulate methane monooxygenase and ammonia monooxygenase may be evolutionarily related. *FEMS Microbiol Lett* **132:** 203-208.

Horn MA, Matthies C, Kusel K, Schramm A, Drake HL (2003). Hydrogenotrophic methanogenesis by moderately acid-tolerant methanogens of a methane-emitting acidic peat. *Appl Environ Microbiol* **69:** 74-83.

Huggett R (2016). *Fundamentals of Geomorphology (3rd edition)*. Routledge: Abingdon, UK.

Hunger S, Schmidt O, Hilgarth M, Horn MA, Kolb S, Conrad R *et al* (2011). Competing formate- and carbon dioxide-utilizing prokaryotes in an anoxic methane-emitting fen soil. *Appl Environ Microbiol* **77:** 3773-3785.

Hurst LD, Merchant AR (2001). High guanine-cytosine content is not an adaptation to high temperature: a comparative analysis amongst prokaryotes. *P Roy Soc B-Biol* Sci **268:** 493-497.

Imachi H, Sakai S (2016). Methanolinea. In: Whitman WB, Rainey F, Kämpfer P, Trujillo M, Chun J, DeVos P et al (eds). *Bergey's Manual of Systematics of Archaea and Bacteria*. John Wiley & Sons. doi: 10.1002/9781118960608.gbm01367.

Inagaki F, Tsunogai U, Suzuki M, Kosaka A, Machiyama H, Takai K *et al* (2004). Characterization of C1-metabolizing prokaryotic communities in methane seep habitats at the Kuroshima Knoll, southern Ryukyu Arc, by analyzing *pmoA*, *mmoX*, *mxaF*, *mcrA*, and 16S rRNA genes. *Appl Environ Microbiol* **70:** 7445-7455.

IPCC (2007). Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB *et al* (eds): Cambridge, United Kingdom and New York, NY, USA.

Islam T, Jensen S, Reigstad LJ, Larsen Ø, Birkeland N-K (2008). Methane oxidation at 55°C and pH 2 by a thermoacidophilic bacterium belonging to the Verrucomicrobia phylum. *Proc Natl Acad Sci USA* **105:** 300-304.

Jaatinen K, Tuittila E-S, Laine J, Yrjälä K, Fritze H (2005). Methane-oxidizing bacteria in a finnish raised mire complex: Effects of site fertility and drainage. *Microb Ecol* **50:** 429-439.

Jabłoński S, Rodowicz P, Łukaszewicz M (2015). Methanogenic archaea database containing physiological and biochemical characteristics. *Int J Syst Evol Microbiol* **65:** 1360-1368.

Jerman V, Metje M, Mandić-Mulec I, Frenzel P (2009). Wetland restoration and methanogenesis: the activity of microbial populations and competition for substrates at different temperatures. *Biogeosciences* **6:** 1127-1138.

Jiang L, Zheng Y, Chen J, Xiao X, Wang F (2011). Stratification of archaeal communities in shallow sediments of the Pearl River Estuary, Southern China. *Antonie Van Leeuwenhoek* **99:** 739-751.

Joabsson A, Christensen TR, Wallén B (1999). Vascular plant controls on methane emissions from northern peatforming wetlands. *Trends Ecol Evol* **14:** 385-388.

Jones RT, Robeson MS, Lauber CL, Hamady M, Knight R, Fierer N (2009). A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. *ISME J* **3:** 442-453.

Joosten H, Clarke D (2002). Wise Use of Mires Peatlands. International Mire Conservation Group and International Peat Society: Devon, UK. p 304.

Joosten H (2009). The Global Peatland CO_2 Picture: peatland status and drainage related emissions in all countries of the world. Wetlands International. p 36.

Joosten H, Tapio-Biström M-L, Tol S (2012). Peatlands: guidance for climate change mitigation through conservation, rehabilitation and sustainable use (2nd edition). Food and Agriculture Organization of the United Nations. p 114.

Joosten H, Brust K, Couwenberg J, Gerner A, Holsten B, Permien T *et al* (2015). MoorFutures - Integration of additional ecosystem services (including biodiversity) into carbon credits–standard, methodology and transferability to other regions. *Federal Agency for Nature Conservation*: Bonn, Germany. p 120.

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

Juottonen H, Galand PE, Yrjala K (2006). Detection of methanogenic archaea in peat: comparison of PCR primers targeting the *mcrA* gene. *Res Microbiol* **157:** 914-921.

Juottonen H, Hynninen A, Nieminen M, Tuomivirta TT, Tuittila ES, Nousiainen H *et al* (2012). Methane-cycling microbial communities and methane emission in natural and restored peatlands. *Appl Environ Microbiol* **78:** 6386-6389.

Jurasinski G, Glatzel S, Hahn J, Koch S, Koch M, Koebsch F: Turn on, fade out-methane exchange in a coastal fen over a period of six years after rewetting. *EGU General Assembly Conference* Vienna, Austria. 2016.

Keller JK, Bridgham SD (2007). Pathways of anaerobic carbon cycling across an ombrotrophic-minerotrophic peatland gradient. *Limnol Oceanogr* **52:** 96-107.

Kelley DS, Karson JA, Fruh-Green GL, Yoerger DR, Shank TM, Butterfield DA *et al* (2005). A serpentinite-hosted ecosystem: the Lost City hydrothermal field. *Science* **307:** 1428-1434.

Kelly CA, Dise NB, Martens CS (1992). Temporal variations in the stable carbon isotopic composition of methane emitted from Minnesota peatlands. *Global Biogeochem Cy* **6:** 263-269.

Kemnitz D, Chin KJ, Bodelier P, Conrad R (2004). Community analysis of methanogenic archaea within a riparian flooding gradient. *Environ Microbiol* **6:** 449-461.

Kettunen A, Kaitala V, Lehtinen A, Lohila A, Alm J, Silvola J *et al* (1999). Methane production and oxidation potentials in relation to water table fluctuations in two boreal mires. *Soil Biol Biochem* **31:** 1741-1749.

Kip N, van Winden JF, Pan Y, Bodrossy L, Reichart GJ, Smolders AJP *et al* (2010). Global prevalence of methane oxidation by symbiotic bacteria in peat-moss ecosystems. *Nature Geoscience* **3:** 617-621.

Kip N, Ouyang WJ, van Winden J, Raghoebarsing A, van Niftrik L, Pol A *et al* (2011). Detection,isolation, and characterization of acidophilic methanotrophs from sphagnum mosses. *Appl Environ Microbiol* **77:** 5643-5654.

Kirschke S, Bousquet P, Ciais P, Saunois M, Canadell JG, Dlugokencky EJ *et al* (2013). Three decades of global methane sources and sinks. *Nature Geoscience* **6:** 813-823.

Knief C (2015). Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on *pmoA* as molecular marker. *Front Microbiol* **6:** doi:10.3389/fmicb.2015. 01346.

Knittel K, Boetius A (2009). Anaerobic oxidation of methane: progress with an unknown process. *Annu Rev Microbiol* **63:** 311-334.

Knittel K, Wegener G, Boetius A (2019). Anaerobic Methane Oxidizers. In: McGenity T (ed). *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*. Springer: Cham. pp 113-132.

Knorr K-H, Lischeid G, Blodau C (2009). Dynamics of redox processes in a minerotrophic fen exposed to a water table manipulation. *Geoderma* **153:** 379-392.

Koch M, Koebsch F, Hahn J, 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:** doi:10.19189/MaP.12015.OMB.19188.

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

Koebsch F, Jurasinski G, Koch M, Hofmann J, Glatzel S (2015). 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.

Kolb S, Knief C, Stubner S, Conrad R (2003). Quantitative detection of methanotrophs in soil by novel *pmoA*-targeted real-time PCR assays. *Appl Environ Microbiol* **69:** 2423-2429.

Kolb S (2009). The quest for atmospheric methane oxidizers in forest soils. *Environ Microbiol Rep* **1:** 336-346.

Komulainen V-M, Nykänen H, Martikainen PJ, Laine J (1998). Short-term effect of restoration on vegetation change and methane emissions from peatlands drained for forestry in southern Finland. *Can J Forest Res* **28:** 402-411.

Kormas KA, Meziti A, Dahlmann A, GJ DEL, Lykousis V (2008). Characterization of methanogenic and prokaryotic assemblages based on *mcrA* and 16*S* rRNA gene diversity in sediments of the Kazan mud volcano (Mediterranean Sea). *Geobiology* **6:** 450-460.

Kotsyurbenko OR, Friedrich MW, Simankova MV, Nozhevnikova AN, Golyshin PN, Timmis KN *et al* (2007). Shift from acetoclastic to H_2-dependent methanogenesis in a west Siberian peat bog at low pH values and isolation of an acidophilic Methanobacterium strain. *Appl Environ Microbiol* **73:** 2344-2348.

Kowalski N, Dellwig O, Beck M, Grunwald M, Dürselen C-D, Badewien TH *et al* (2012). A comparative study of manganese dynamics in the water column and sediments of intertidal systems of the North Sea. *Estuar Coast Shelf Sci* **100:** 3-17.

Krause S, Luke C, Frenzel P (2012). Methane source strength and energy flow shape methanotrophic communities in oxygen-methane counter-gradients. *Environ Microbiol Rep* **4:** 203-208.

Lang K, Schuldes J, Klingl A, Poehlein A, Daniel R, Brune A (2015). New mode of energy metabolism in the seventh order of methanogens as revealed by comparative genome analysis of "*Candidatus Methanoplasma termitum*". *Appl Environ Microbiol* **81:** 1338-1352.

Langenheder S, Székely AJ (2011). Species sorting and neutral processes are both important during the initial assembly of bacterial communities. *ISME J* **5:** 1086-1094.

Lazar CS, Dinasquet J, L'Haridon S, Pignet P, Toffin L (2011a). Distribution of anaerobic methane-oxidizing and sulfate-reducing communities in the G11 Nyegga pockmark, Norwegian Sea. *Antonie Van Leeuwenhoek* **100:** 639-653.

Lazar CS, Parkes RJ, Cragg BA, L'Haridon S, Toffin L (2011b). Methanogenic diversity and activity in hypersaline sediments of the centre of the Napoli mud volcano, Eastern Mediterranean Sea. *Environ Microbiol* **13:** 2078-2091.

Lazar CS, John Parkes R, Cragg BA, L'Haridon S, Toffin L (2012). Methanogenic activity and diversity in the centre of the Amsterdam Mud Volcano, Eastern Mediterranean Sea. *FEMS Microbiol Ecol* **81:** 243-254.

Le Mer J, Roger P (2001). Production, oxidation, emission and consumption of methane by soils: a review. *Eur J Soil Biol* **37:** 25-50.

Li Q, Wang F, Chen Z, Yin X, Xiao X (2012). Stratified active archaeal communities in the sediments of Jiulong River estuary, China. *Front Microbiol* **3:** 311.

Lieberman RL, Rosenzweig AC (2004). Biological methane oxidation: Regulation, biochemistry, and active site structure of particulate methane monooxygenase. *Crit Rev Biochem Mol* **39:** 147-164.

Liebner S, Schwarzenbach SP, Zeyer J (2012). Methane emissions from an alpine fen in central Switzerland. *Biogeochemistry* **109:** 287-299.

Liebner S, Ganzert L, Kiss A, Yang S, Wagner D, Svenning MM (2015). Shifts in methanogenic community composition and methane fluxes along the degradation of discontinuous permafrost. *Front Microbiol* **6:** 356.

Liu DY, Ding WX, Jia ZJ, Cai ZC (2011). Relation between methanogenic archaea and methane production potential in selected natural wetland ecosystems across China. *Biogeosciences* **8:** 329-338.

Liu J, Liu M, Wu M, Jiang C, Chen X, Cai Z *et al* (2018). Soil pH rather than nutrients drive changes in microbial community following long-term fertilization in acidic Ultisols of southern China. *J Soil Sediment* **18:** 1853-1864.

Liu Y, Whitman WB (2008). Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Ann N Y Acad Sci* **1125:** 171-189.

Liu Y (2010). Taxonomy of methanogens. *Handbook of hydrocarbon and lipid microbiology*. Springer. pp 547-558.

Liu Y, Priscu JC, Xiong J, Conrad R, Vick-Majors T, Chu H *et al* (2016). Salinity drives archaeal distribution patterns in high altitude lake sediments on the Tibetan Plateau. *FEMS Microbiol Ecol* **92:** fiw033.

Lloyd KG, Lapham L, Teske A (2006). An anaerobic methane-oxidizing community of ANME-1b archaea in hypersaline Gulf of Mexico sediments. *Appl Environ Microbiol* **72:** 7218-7230.

Lofton DD, Whalen SC, Hershey AE (2014). Effect of temperature on methane dynamics and evaluation of methane oxidation kinetics in shallow Arctic Alaskan lakes. *Hydrobiologia* **721:** 209-222.

Logares R, Lindstrom ES, Langenheder S, Logue JB, Paterson H, Laybourn-Parry J *et al* (2013). Biogeography of bacterial communities exposed to progressive long-term environmental change. *ISME J* **7:** 937-948.

Lomolino MV, Riddle BR, Whittaker RJ (2017). *Biogeography (Fifth Edition)*. Oxford University Press: Sunderland, MA.

Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hector A *et al* (2001). Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science* **294:** 804-808.

Lozupone CA, Knight R (2007). Global patterns in bacterial diversity. *Proc Natl Acad Sci USA* **104:** 11436-11440.

Lueders T, Chin K-J, Conrad R, Friedrich M (2001). Molecular analyses of methyl-coenzyme M reductase α-subunit (*mcrA*) genes in rice field soil and enrichment cultures reveal the methanogenic phenotype of a novel archaeal lineage. *Environ Microbiol* **3:** 194-204.

Luesken FA, Zhu BL, van Alen TA, Butler MK, Diaz MR, Song B *et al* (2011). *pmoA* primers for detection of anaerobic methanotrophs. *Appl Environ Microbiol* **77:** 3877-3880.

Lüke C, Krause S, Cavigiolo S, Greppi D, Lupotto E, Frenzel P (2010). Biogeography of wetland rice methanotrophs. *Environ Microbiol* **12:** 862-872.

Lüke C, Frenzel P (2011). Potential of *pmoA* amplicon pyrosequencing for methanotroph diversity studies. *Appl Environ Microbiol* **77:** 6305-6309.

Luton PE, Wayne JM, Sharp RJ, Riley PW (2002). The *mcrA* gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. *Microbiology* **148:** 3521-3530.

Lutz S, Anesio AM, Raiswell R, Edwards A, Newton RJ, Gill F *et al* (2016). The biogeography of red snow microbiomes and their role in melting arctic glaciers. *Nat Commun* **7:** 11968.

Mackelprang R, Waldrop MP, DeAngelis KM, David MM, Chavarria KL, Blazewicz SJ *et al* (2011). Metagenomic analysis of a permafrost microbial community reveals a rapid response to thaw. *Nature* **480:** 368-371.

Mackelprang R, Saleska SR, Jacobsen CS, Jansson JK, Taş N (2016). Permafrost meta-omics and climate change. *Annu Rev Earth Planet Sci* **44:** 439-462.

Malik AA, Puissant J, Buckeridge KM, Goodall T, Jehmlich N, Chowdhury S *et al* (2018). Land use driven change in soil pH affects microbial carbon cycling processes. *Nat Commun* **9:** 3591.

Martin M (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal* **17:** 3. doi: https://doi.org/10.14806/ej.17.1.200

Martiny JB, Bohannan BJ, Brown JH, Colwell RK, Fuhrman JA, Green JL *et al* (2006). Microbial biogeography: putting microorganisms on the map. *Nat Rev Microbiol* **4:** 102-112.

Martiny JB, Eisen JA, Penn K, Allison SD, Horner-Devine MC (2011). Drivers of bacterial β-diversity depend on spatial scale. *Proc Natl Acad Sci USA* **108:** 7850-7854.

Maxfield PJ, Hornibrook ERC, Evershed RP (2008). Acute impact of agriculture on high-affinity methanotrophic bacterial populations. *Environmen Microbiol* **10**: 1917-1924.

McArdle BH, Anderson MJ (2001). Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* **82**: 290-297.

McCalley CK, Woodcroft BJ, Hodgkins SB, Wehr RA, Kim EH, Mondav R *et al* (2014). Methane dynamics regulated by microbial community response to permafrost thaw. *Nature* **514:** 478-481.

McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A *et al* (2011). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J* **6:** 610-618.

McGenity T (2010). Methanogens and methanogenesis in hypersaline environments. In: K.N. T (ed). *Handbook of Hydrocarbon and Lipid Microbiology*. Springer: Berlin, Heidelberg. pp 665-680.

McLusky DS, Elliott M (2004). *The estuarine ecosystem: ecology, threats and management*. Oxford University Press: Oxford, Newyork.

Megonigal JP, Hines M, Visscher P (2004). Anaerobic metabolism: linkages to trace gases and aerobic processes. In: Schlesinger WH (ed). *Biogeochemistry*. Elsevier-Pergamon: Oxford, UK. pp 317-424.

Melton JR, Wania R, Hodson EL, Poulter B, Ringeval B, Spahni R *et al* (2013). Present state of global wetland extent and wetland methane modelling: conclusions from a model inter-comparison project (WETCHIMP). *Biogeosciences* **10:** 753-788.

Merila P, Galand PE, Fritze H, Tuittila ES, Kukko-Oja K, Laine J *et al* (2006). Methanogen communities along a primary succession transect of mire ecosystems. *FEMS Microbiol Ecol* **55:** 221-229.

Meyer KM, Memiaghe H, Korte L, Kenfack D, Alonso A, Bohannan BJ (2018). Why do microbes exhibit weak biogeographic patterns? *ISME J* **12:** 1404-1413.

Middelburg JJ, Nieuwenhuize J, Iversen N, Høgh N, De Wilde H, Helder W *et al* (2002). Methane distribution in European tidal estuaries. *Biogeochemistry* **59:** 95-119.

Milferstedt K, Youngblut ND, Whitaker RJ (2010). Spatial structure and persistence of methanogen populations in humic bog lakes. *ISME J* **4:** 764-776.

Minke M, Augustin J, Burlo A, Yarmashuk T, Chuvashova H, Thiele A *et al* (2016). Water level, vegetation composition, and plant productivity explain greenhouse gas fluxes in temperate cutover fens after inundation. *Biogeosciences* **13:** 3945-3970.

Minkkinen K, Korhonen R, Savolainen I, Laine J (2002). Carbon balance and radiative forcing of Finnish peatlands 1900-2100 - the impact of forestry drainage. *Global Change Biol* **8:** 785-799.

Minkkinen K, Laine J (2006). Vegetation heterogeneity and ditches create spatial variability in methane fluxes from peatlands drained for forestry. *Plant Soil* **285:** 289-304.

Mitsch WJ, Gosselink JG, Zhang L, Anderson CJ (2009). *Wetland Ecosystems*. John Wiley & Sons.

Miyazaki J, Higa R, Toki T, Ashi J, Tsunogai U, Nunoura T *et al* (2009). Molecular characterization of potential nitrogen fixation by anaerobic methane-oxidizing archaea in the methane seep sediments at the number 8 Kumano Knoll in the Kumano Basin, offshore of Japan. *Appl Environ Microbiol* **75:** 7153-7162.

Montoya L, Lozada-Chavez I, Amils R, Rodriguez N, Marin I (2011). The sulfate-rich and extreme saline sediment of the ephemeral tirez lagoon: a biotope for acetoclastic sulfate-reducing bacteria and hydrogenotrophic methanogenic archaea. *Int J Microbiol* **2011:** 753758.

Mosier AC, Francis CA (2008). Relative abundance and diversity of ammonia-oxidizing archaea and bacteria in the San Francisco Bay estuary. *Environ Microbiol* **10:** 3002-3016.

Murrell JC, Gilbert B, McDonald IR (2000). Molecular biology and regulation of methane monooxygenase. *Arch Microbiol* **173:** 325-332.

Myhre G, Shindell D, Bréon F-M, Collins W, Fuglestvedt J, Huang J *et al* (2013). Anthropogenic and natural radiative forcing. *Climate Change* **423:** 658-740.

Narihiro T, Hori T, Nagata O, Hoshino T, Yumoto I, Kamagata Y (2011). The impact of aridification and vegetation type on changes in the community structure of methane-cycling microorganisms in Japanese wetland soils. *Biosci Biotechnol Biochem* **75:** 1727-1734.

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

Nelson MB, Martiny AC, Martiny JB (2016). Global biogeography of microbial nitrogen-cycling traits in soil. *Proc Natl Acad Sci USA* **113:** 8033-8040.

Nemergut DR, Costello EK, Hamady M, Lozupone C, Jiang L, Schmidt SK *et al* (2011). Global patterns in the biogeography of bacterial taxa. *Environ Microbiol* **13:** 135-144.

Newberry CJ, Webster G, Cragg BA, Parkes RJ, Weightman AJ, Fry JC (2004). Diversity of prokaryotes and methanogenesis in deep subsurface sediments from the Nankai Trough, Ocean Drilling Program Leg 190. *Environ Microbiol* **6:** 274-287.

Nicol GW, Leininger S, Schleper C, Prosser JI (2008). The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environ Microbiol* **10:** 2966-2978.

Nobu MK, Narihiro T, Kuroda K, Mei R, Liu WT (2016). Chasing the elusive Euryarchaeota class WSA2: genomes reveal a uniquely fastidious methyl-reducing methanogen. *ISME J* **10:** 2478-2487.

Nunoura T, Takaki Y, Shimamura S, Kakuta J, Kazama H, Hirai M *et al* (2016). Variance and potential niche separation of microbial communities in subseafloor sediments off Shimokita Peninsula, Japan. *Environ Microbiol* **18:** 1889-1906.

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

Oakley BB, Carbonero F, Dowd SE, Hawkins RJ, Purdy KJ (2012). Contrasting patterns of niche partitioning between two anaerobic terminal oxidizers of organic matter. *ISME J* **6:** 905-914.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara R *et al* (2013). vegan: Community Ecology Package. R Package Version. 2.0-10. URL: https://CRAN.R-project.org/package=vegan.

Oloo F, Valverde A, Quiroga MV, Vikram S, Cowan D, Mataloni G (2016). Habitat heterogeneity and connectivity shape microbial communities in South American peatlands. *Sci Rep* **6:** 25712.

Op den Camp HJM, Islam T, Stott MB, Harhangi HR, Hynes A, Schouten S *et al* (2009). Environmental, genomic and taxonomic perspectives on methanotrophic *Verrucomicrobia*. *Environ Microbiol Rep* **1:** 293-306.

Oren A, Garrity GM (2015). List of new names and new combinations previously effectively, but not validly, published. *Int J Syst Evol Microbiol* **65:** 2017-2025.

Ortiz-Llorente MJ, Alvarez-Cobelas M (2012). Comparison of biogenic methane emissions from unmanaged estuaries, lakes, oceans, rivers and wetlands. *Atmos Environ* **59:** 328-337.

Osudar R, Klings KW, Wagner D, Bussmann I (2018). Effect of salinity on microbial methane oxidation in freshwater and marine environments. *Aquat Microb Ecol* **80:** 181-192.

Oswald K, Graf JS, Littmann S, Tienken D, Brand A, Wehrli B *et al* (2017). *Crenothrix* are major methane consumers in stratified lakes. *ISME J* **11:** 2124-2140.

Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W, Christ R *et al* (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*. IPCC.

Paradis E, Claude J, Strimmer K (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* **20:** 289-290.

Parkes RJ, Webster G, Cragg BA, Weightman AJ, Newberry CJ, Ferdelman TG *et al* (2005). Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. *Nature* **436:** 390-394.

Pattnaik P, Mishra S, Bharati K, Mohanty S, Sethunathan N, Adhya T (2000). Influence of salinity on methanogenesis and associated microflora in tropical rice soils. *Microbiol Res* **155:** 215-220.

Paul K, Nonoh JO, Mikulski L, Brune A (2012). "Methanoplasmatales," Thermoplasmatales-related archaea in termite guts and other environments, are the seventh order of methanogens. *Appl Environ Microbiol* **78:** 8245-8253.

Peltoniemi K, Laiho R, Juottonen H, Bodrossy L, Kell DK, Minkkinen K *et al* (2016). Responses of methanogenic and methanotrophic communities to warming in varying moisture regimes of two boreal fens. *Soil Biol Biochem* **97:** 144-156.

Pittock J, Finlayson M, Arthington A, Roux D, Matthews J, H B et al (2015). Managing freshwater, river, wetland and estuarine protected areas. In: Worboys GL, Lockwood M, Kothari A, Feary S, Pulsford I (eds). Protected Area Governance and Management. ANU Press: Canberra. pp 569–608

Poffenbarger HJ, Needelman BA, Megonigal JP (2011). Salinity influence on methane emissions from tidal marshes. *Wetlands* **31:** 831-842.

Porsch K, Wirth B, Toth EM, Schattenberg F, Nikolausz M (2015). Characterization of wheat straw-degrading anaerobic alkali-tolerant mixed cultures from soda lake sediments by molecular and cultivation techniques. *Microb Biotechnol* **8:** 801-814.

Potter EG, Bebout BM, Kelley CA (2009). Isotopic composition of methane and inferred methanogenic substrates along a salinity gradient in a hypersaline microbial mat system. *Astrobiology* **9:** 383-390.

Putkinen A, Tuittila E-S, Siljanen HMP, Bodrossy L, Fritze H (2018). Recovery of methane turnover and the associated microbial communities in restored cutover peatlands is strongly linked with increasing Sphagnum abundance. *Soil Biol Biochem* **116:** 110-119.

Qiu QF, Noll M, Abraham WR, Lu YH, Conrad R (2008). Applying stable isotope probing of phospholipid fatty acids and rRNA in a Chinese rice field to study activity and composition of the methanotrophic bacterial communities in situ. *ISME J* **2:** 602-614.

Quast C, Pruesse E, Gerken J, Peplies J, Yarza P, Yilmaz P *et al* (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* **41:** D590-D596.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013.

Raghoebarsing AA, Pol A, van de Pas-Schoonen KT, Smolders AJP, Ettwig KF, Rijpstra WIC *et al* (2006). A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature* **440:** 918-921.

Rahalkar M, Schink B (2007). Comparison of aerobic methanotrophic communities in littoral and profundal sediments of Lake Constance by a molecular approach. *Appl Environ Microbiol* **73:** 4389-4394.

Ramette A, Tiedje JM (2007). Biogeography: An emerging cornerstone for understanding prokaryotic diversity, ecology, and evolution. *Microb Ecol* **53:** 197-207.

Rath KM, Fierer N, Murphy DV, Rousk J (2019). Linking bacterial community composition to soil salinity along environmental gradients. *ISME J* **13:** 836-846.

Reed AJ, Dorn R, Van Dover CL, Lutz RA, Vetriani C (2009). Phylogenetic diversity of methanogenic, sulfate-reducing and methanotrophic prokaryotes from deep-sea hydrothermal vents and cold seeps. *Deep-Sea Res (2 Top Stud Oceanogr)* **56:** 1665-1674.

Ren B, Hu Y, Chen B, Zhang Y, Thiele J, Shi R *et al* (2018). Soil pH and plant diversity shape soil bacterial community structure in the active layer across the latitudinal gradients in continuous permafrost region of Northeastern China. *Sci Rep* **8:** 5619.

Roberts D (2016). labdsv: ordination and multivariate analysis for ecology. R package version 1.8-0. URL: https://CRAN.R-project.org/package=labdsv.

Romanovsky VE, Osterkamp TE (1997). Thawing of the active layer on the coastal plain of the Alaskan Arctic. *Permafrost Periglac* **8:** 1-22.

Rooney-Varga JN, Giewat MW, Duddleston KN, Chanton JP, Hines ME (2007). Links between archaeal community structure, vegetation type and methanogenic pathway in Alaskan peatlands. *FEMS Microbiol Ecol* **60:** 240-251.

Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (2014). *The Prokaryotes: Other Major Lineages of Bacteria and The Archaea*. Springer Berlin Heidelberg.

Roulet NT, Lafleur PM, Richard PJ, Moore TR, Humphreys ER, Bubier J (2007). Contemporary carbon balance and late Holocene carbon accumulation in a northern peatland. *Global Change Biol* **13:** 397-411.

Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG *et al* (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J* **4:** 1340-1351.

Roussel EG, Sauvadet A-L, Allard J, Chaduteau C, Richard P, Bonavita M-AC *et al* (2009). Archaeal methane cycling communities associated with gassy subsurface sediments of Marennes-Oléron Bay (France). *Geomicrobiol J* **26:** 31-43.

Roussel EG, Konn C, Charlou JL, Donval JP, Fouquet Y, Querellou J *et al* (2011). Comparison of microbial communities associated with three Atlantic ultramafic hydrothermal systems. *FEMS Microbiol Ecol* **77:** 647-665.

Ruff SE, Biddle JF, Teske AP, Knittel K, Boetius A, Ramette A (2015). Global dispersion and local diversification of the methane seep microbiome. *Proc Natl Acad Sci USA* **112:** 4015-4020.

Saarnio S, Winiwarter W, Leitão J (2009). Methane release from wetlands and watercourses in Europe. *Atmos Environ* **43:** 1421-1429.

Santana PB, Ghilardi Junior R, Alves CN, Silva JL, McCulloch JA, Schneider MPC *et al* (2012). Diversity and three-dimensional structures of the alpha Mcr of the methanogenic Archaea from the anoxic region of Tucuruí Lake, in Eastern Brazilian Amazonia. *Genet Mol Biol* **35:** 126-133.

Sas JC (2009). A geomicrobiological investigation of sub-surface mud volcano sediments from the Gulf of Cadiz. Doctor Thesis, Cardiff University.

Saunois M, Jackson R, Bousquet P, Poulter B, Canadell J (2016). The growing role of methane in anthropogenic climate change. *Environ Res Lett* **11:** 120207.

Schaefer H, Fletcher SEM, Veidt C, Lassey KR, Brailsford GW, Bromley TM *et al* (2016). A 21st-century shift from fossil-fuel to biogenic methane emissions indicated by $^{13}CH_4$. *Science* **352:** 80-84.

Schauer R, Bienhold C, Ramette A, Harder J (2009). Bacterial diversity and biogeography in deep-sea surface sediments of the South Atlantic Ocean. *ISME J* **4:** 159-170.

Schemel LE (2001). Simplified conversions between specific conductance and salinity units for use with data from monitoring stations. *Interagency Ecol Program Newslett* **14:** 17-18.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB *et al* (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75:** 7537-7541.

Schönfeld-Bockholt R, Roth D, Dittmann L: Teilflächenbezogene ökologische und futterwirtschaftliche Beurteilung des Grünlandes im Naturschutzgebiet Heiligensee und Hütelmoor. *Effiziente Nutzung von Grünland als Ressource für die Milch- und Fleischproduktion Mitteilungen der Arbeitsgemeinschaft Grünland und Futterbau*; Aug 28-30; Zollikofen, Schweiz. 2008.

Schuur EAG, Abbott B, Network PC (2011). High risk of permafrost thaw. *Nature* **480:** 32-33.

Segers R (1998). Methane production and methane consumption: a review of processes underlying wetland methane fluxes. *Biogeochemistry* **41:** 23-51.

Semrau JD, DiSpirito AA, Yoon S (2010). Methanotrophs and copper. *FEMS Microbiol Rev* **34:** 496-531.

Shakhova N, Semiletov I (2007). Methane release and coastal environment in the East Siberian Arctic shelf. *J Mar Sys* **66:** 227-243.

She C, Tong C (2012). Vertical distribution of methanogen community structures in Phragmites australis marsh soil in the Min river estuary. *Acta Ecologica Sinica* **32:** 5299-5308 (in Chinese).

Silvius M, Joosten H, Opdam S, Parish F, Sirin A, Charman D *et al* (2008). Assessment on Peatlands, Biodiversity and Climate Change: Main Report. Global Environment Center, Kuala Lumpur and Wetlands International: Wageningen. pp 20-38.

Sofi MH, Gudi R, Karumuthil-Melethil S, Perez N, Johnson BM, Vasu C (2014). pH of drinking water influences the composition of gut microbiome and type 1 diabetes incidence. *Diabetes* **63:** 632-644.

Söllinger A, Schwab C, Weinmaier T, Loy A, Tveit AT, Schleper C *et al* (2015). Phylogenetic and genomic analysis of Methanomassiliicoccales in wetlands and animal intestinal tracts reveals clade-specific habitat preferences. *FEMS Microbiol Ecol* **92:** fiv149.

Statham PJ (2012). Nutrients in estuaries - An overview and the potential impacts of climate change. *Sci Total Environ* **434:** 213-227.

Steenbergh AK, Meima MM, Kamst M, Bodelier PLE (2010). Biphasic kinetics of a methanotrophic community is a combination of growth and increased activity per cell. *FEMS Microbiol Ecol* **71:** 12-22.

Steffenhagen P, Zak D, Schulz K, Timmermann T, Zerbe S (2012). Biomass and nutrient stock of submersed and floating macrophytes in shallow lakes formed by rewetting of degraded fens. *Hydrobiologia* **692:** 99-109.

Steinberg LM, Regan JM (2008). Phylogenetic comparison of the methanogenic communities from an acidic, oligotrophic fen and an anaerobic digester treating municipal wastewater sludge. *Appl Environ Microbiol* **74:** 6663-6671.

Steinberg LM, Regan JM (2009). *mcrA*-targeted real-time quantitative PCR method to examine methanogen communities. *Appl Environ Microbiol* **75:** 4435-4442.

Stoecker K, Bendinger B, Schoning B, Nielsen PH, Nielsen JL, Baranyi C *et al* (2006). Cohn's *Crenothrix* is a filamentous methane oxidizer with an unusual methane monooxygenase. *P Natl Acad Sci USA* **103:** 2363-2367.

Stoeva MK, Aris-Brosou S, Chetelat J, Hintelmann H, Pelletier P, Poulain AJ (2014). Microbial community structure in lake and wetland sediments from a high arctic polar desert revealed by targeted transcriptomics. *Plos One* **9:** e89531.

Stott PA, Sutton RT, Smith DM (2008). Detection and attribution of Atlantic salinity changes. *Geophys Res Lett* **35:** doi:10.1029/2008gl035874.

Sun CL, Brauer SL, Cadillo-Quiroz H, Zinder SH, Yavitt JB (2012). Seasonal changes in methanogenesis and methanogenic community in three peatlands, New York State. *Front Microbiol* **3:** 81.

Surakasi VP, Wani A, Shouche Y, Ranade D (2007). Phylogenetic analysis of methanogenic enrichment cultures obtained from Lonar Lake in India: isolation of *Methanocalculus* sp. and *Methanoculleus* sp. *Microb Ecol* **54**: 697-704.

Takai K, Horikoshi K (2000). Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. *Appl Environ Microbiol* **66:** 5066-5072.

Takai K, Nakamura K, Toki T, Tsunogai U, Miyazaki M, Miyazaki J *et al* (2008). Cell proliferation at 122°C and isotopically heavy CH_4 production by a hyperthermophilic methanogen under high-pressure cultivation. *Proc Natl Acad Sci USA* **105:** 10949-10954.

Taketani RG, Yoshiura CA, Dias AC, Andreote FD, Tsai SM (2010). Diversity and identification of methanogenic archaea and sulphate-reducing bacteria in sediments from a pristine tropical mangrove. *Antonie Van Leeuwenhoek* **97:** 401-411.

Tavormina PL, Orphan VJ, Kalyuzhnaya MG, Jetten MSM, Klotz MG (2011). A novel family of functional operons encoding methane/ammonia monooxygenase-related proteins in gammaproteobacterial methanotrophs. *Environ Microbiol Rep* **3:** 91-100.

Thauer RK, Kaster A-K, Seedorf H, Buckel W, Hedderich R (2008). Methanogenic archaea: ecologically relevant differences in energy conservation. *Nat Rev Microbiol* **6:** 579-591.

Tilman D, Isbell F, Cowles JM (2014). Biodiversity and ecosystem functioning. *Annu Rev Ecol Evol Syst* **45:** 471-493.

Tringe SG, Hugenholtz P (2008). A renaissance for the pioneering 16S rRNA gene. *Curr Opin Microbiol* **11:** 442-446.

Trotsenko YA, Murrell JC (2008). Metabolic aspects of aerobic obligate methanotrophy. In: Laskin AI, Sariaslani S, Gadd GM (eds). *Advance in Applied Microbiology*. Academic Press: London. pp 183-229.

Tsubota J, Eshinimaev BT, Khmelenina VN, Trotsenko YA (2005). *Methylothermus thermalis* gen. nov., sp. nov., a novel moderately thermophilic obligate methanotroph from a hot spring in Japan. *Int J Syst Evol Microbiol* **55:** 1877-1884.

Tuittila E-S, Komulainen V-M, Vasander H, Nykänen H, Martikainen PJ, Laine J (2000). Methane dynamics of a restored cut-away peatland. *Global Change Biol* **6:** 569-581.

Tveit AT, Urich T, Frenzel P and Svenning MM (2015). Metabolic and trophic interactions modulate methane production by Arctic peat microbiota in response to warming. *Proc Natl Acad Sci USA* **112**: 2507-2516.

Tveit AT, Hestnes AG, Robinson SL, Schintlmeister A, Dedysh SN, Jehmlich N *et al* (2019). Widespread soil bacterium that oxidizes atmospheric methane. *Proc Natl Acad Sci USA* **116:** 8515-8524.

Ulrich W, Hajdamowicz I, Zalewski M, Stańska M, Ciurzycki W, Tykarski P (2010). Species assortment or habitat filtering: a case study of spider communities on lake islands. *Ecol Res* **25:** 375-381.

Urbanová Z, Picek T, Bárta J (2011). Effect of peat re-wetting on carbon and nutrient fluxes, greenhouse gas production and diversity of methanogenic archaeal community. *Ecol Engineer* **37:** 1017-1026.

Urbanová Z, Bárta J, Picek T (2013). Methane emissions and methanogenic archaea on pristine, drained and restored mountain peatlands, Central Europe. *Ecosystems* **16:** 664-677.

van Bodegom P, Stams F, Mollema L, Boeke S, Leffelaar P (2001). Methane oxidation and the competition for oxygen in the rice rhizosphere. *Appl Environ Microbiol* **67:** 3586-3597.

van der Gon HACD, Neue H-U (1995). Methane emission from a wetland rice field as affected by salinity. *Plant Soil* **170:** 307-313.

van Dijk G, Smolders AJP, Loeb R, Bout A, Roelofs JGM, Lamers LPM (2015). Salinization of coastal freshwater wetlands; effects of constant versus fluctuating salinity on sediment biogeochemistry. *Biogeochemistry* **126:** 71-84.

Vanselow-Algan M, Schmidt SR, Greven M, Fiencke C, Kutzbach L, Pfeiffer EM (2015). High methane emissions dominated annual greenhouse gas balances 30 years after bog rewetting. *Biogeosciences* **12:** 4361-4371.

Vanwonterghem I, Evans PN, Parks DH, Jensen PD, Woodcroft BJ, Hugenholtz P *et al* (2016). Methylotrophic methanogenesis discovered in the archaeal phylum Verstraetearchaeota. *Nat Microbiol* **1:** 16170.

Várallyay G (1994). *Climate Change, Soil Salinity and Alkalinity*. Springer: Berlin, Heidelberg.

Vasander H, Kettunen A (2006). Carbon in boreal peatlands. In: Wieder R, Vitt D (eds). *Boreal Peatland Ecosystems*. Springer: Berlin, Heidelberg. pp 165-194.

Ver Eecke HC, Butterfield DA, Huber JA, Lilley MD, Olson EJ, Roe KK *et al* (2012). Hydrogen-limited growth of hyperthermophilic methanogens at deep-sea hydrothermal vents. *Proc Natl Acad Sci USA* **109:** 13674-13679.

Vigneron A, Cruaud P, Roussel EG, Pignet P, Caprais JC, Callac N *et al* (2014). Phylogenetic and functional diversity of microbial communities associated with subsurface sediments of the Sonora Margin, Guaymas Basin. *Plos One* **9:** e104427.

Voigtländer U, Schmidt J, Scheller W (1996). Pflege-und Entwicklungsplan NSG Heiligensee und Hütelmoor. *SALIX, Büro für Landschaftsplanung Waren, Teterow, Germany*.

von Mering C, Hugenholtz P, Raes J, Tringe SG, Doerks T, Jensen LJ *et al* (2007). Quantitative phylogenetic assessment of microbial communities in diverse environments. *Science* **315:** 1126-1130.

Waddington J, Warner K, Kennedy G (2002). Cutover peatlands: a persistent source of atmospheric CO_2. *Global Biogeochem Cy* **16:** 1-7.

Waddington J, Day S (2007). Methane emissions from a peatland following restoration. *J Geophys Res-Biogeo* **112:** G03018.

Wagner D, Liebner S (2009). Global warming and carbon dynamics in permafrost soils: methane production and oxidation. In: Margesin R (ed). *Permafrost Soils*. Springer: Berlin. pp 219-236.

Wagner D (2017). Effect of varying soil water potentials on methanogenesis in aerated marshland soils. *Sci Rep* **7:** 14706.

Wang PL, Chiu YP, Cheng TW, Chang YH, Tu WX, Lin LH (2014). Spatial variations of community structures and methane cycling across a transect of Lei-Gong-Hou mud volcanoes in eastern Taiwan. *Front Microbiol* **5:** doi:10.3389/fmicb.2014.00121.

Wang ZP, DeLaune RD, Patrick WH, Masscheleyn PH (1993). Soil redox and ph effects on methane production in a flooded rice soil. *Soil Sci Soc Am J* **57:** 382-385.

Weiher E, Keddy P (2001). *Ecological assembly rules: perspectives, advances, retreats*. Cambridge University Press: Cambridge, UK.

Weisner E, Schernewski G (2013). Adaptation to climate change: A combined coastal protection and re-alignment scheme in a Baltic tourism region. *J Coastal Res* **65:** 1963-1969.

Wen X, Yang S, Liebner S (2016). Evaluation and update of cutoff values for methanotrophic *pmoA* gene sequences. *Arch Microbiol* **198:** 1-8.

Wen X, Yang S, Horn F, Winkel M, Wagner D, Liebner S (2017). Global biogeographic analysis of methanogenic archaea identifies community-shaping environmental factors of natural environments. *Front Microbiol* **8:** 1339.

Wen X, Unger V, Jurasinski G, Koebsch F, Horn F, Rehder G *et al* (2018). Predominance of methanogens over methanotrophs in rewetted fens characterized by high methane emissions. *Biogeosciences* **15:** 6519-6536.

Whitaker RJ, Grogan DW, Taylor JW (2003). Geographic barriers isolate endemic populations of hyperthermophilic archaea. *Science* **301:** 976-978.

Whittenbury R, Davies SL, Davey JF (1970). Exospores and cysts formed by methane-utilizing bacteria. *Microbiology* **61:** 219-226.

Wickham H (2009). ggplot2: Elegant Graphics for Data Analysis. 2009. URL*:* http://ggplot2.org.

Wickham H (2016). *ggplot2: elegant graphics for data analysis*. Springer.

Wilhelm E, Battino R, Wilcock RJ (1977). Low-pressure solubility of gases in liquid water. *Chem Rev* **77:** 219-262.

Wilson D, Alm J, Laine J, Byrne KA, Farrell EP, Tuittila ES (2009). Rewetting of cutaway peatlands: Are we re-creating hot spots of methane emissions? *Restor Ecol* **17:** 796-806.

Wilson D, Blain D, Couwenberg J, Evans C, Murdiyarso D, Page S *et al* (2016). Greenhouse gas emission factors associated with rewetting of organic soils. *Mires Peat* **17:** doi:10.19189/MaP.12016.OMB.19222.

Winkel M, Mitzscherling J, Overduin PP, Horn F, Winterfeld M, Rijkers R *et al* (2018). Anaerobic methanotrophic communities thrive in deep submarine permafrost. *Sci Rep* **8:** 1291.

Wu Y, Zeng J, Zhu Q, Zhang Z, Lin X (2017). pH is the primary determinant of the bacterial community structure in agricultural soils impacted by polycyclic aromatic hydrocarbon pollution. *Sci Rep* **7:** 40093.

Yang J, Ma La, Jiang H, Wu G, Dong H (2016a). Salinity shapes microbial diversity and community structure in surface sediments of the Qinghai-Tibetan Lakes. *Sci Rep* **6:** 25078.

Yang S, Liebner S, Alawi M, Ebenhöh O, Wagner D (2014). Taxonomic database and cut-off value for processing *mcrA* gene 454 pyrosequencing data by MOTHUR. *J Microbiol Methods* **103:** 3-5.

Yang S, Wen X, Liebner S (2016b). *pmoA* gene reference database (fasta-formatted sequences and taxonomy). GFZ Data Services: Potsdam.

Yang S, Liebner S, Winkel M, Alawi M, Horn F, Dörfer C *et al* (2017). In-depth analysis of core methanogenic communities from high elevation permafrost-affected wetlands. *Soil Biol Biochem* **111:** 66-77.

Yavitt JB, Yashiro E, Cadillo-Quiroz H, Zinder SH (2011). Methanogen diversity and community composition in peatlands of the central to northern Appalachian Mountain region, North America. *Biogeochemistry* **109:** 117-131.

Ye R, Jin Q, Bohannan B, Keller JK, McAllister SA, Bridgham SD (2012). pH controls over anaerobic carbon mineralization, the efficiency of methane production, and methanogenic pathways in peatlands across an ombrotrophic–minerotrophic gradient. *Soil Biol Biochem* **54:** 36-47.

Yoshioka H, Maruyama A, Nakamura T, Higashi Y, Fuse H, Sakata S *et al* (2010). Activities and distribution of methanogenic and methane-oxidizing microbes in marine sediments from the Cascadia Margin. *Geobiology* **8:** 223-233.

Youngblut ND, Dell'aringa M, Whitaker RJ (2014). Differentiation between sediment and hypolimnion methanogen communities in humic lakes. *Environ Microbiol* **16:** 1411-1423.

Yrjälä KIM, Tuomivirta T, Juottonen H, Putkinen A, Lappi K, Tuittila E-S *et al* (2011). CH_4 production and oxidation processes in a boreal fen ecosystem after long-term water table drawdown. *Global Change Biol* **17:** 1311-1320.

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.

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.

Zeleke J, Lu SL, Wang JG, Huang JX, Li B, Ogram AV *et al* (2013a). Methyl coenzyme M reductase A (*mcrA*) gene-based investigation of methanogens in the mudflat sediments of Yangtze River estuary, China. *Microb Ecol* **66:** 257-267.

Zeleke J, Sheng Q, Wang JG, Huang MY, Xia F, Wu JH *et al* (2013b). Effects of Spartina alterniflora invasion on the communities of methanogens and sulfate-reducing bacteria in estuarine marsh sediments. *Front Microbiol* **4:** 243.

Zhang G, Tian J, Jiang N, Guo X, Wang Y, Dong X (2008a). Methanogen community in Zoige wetland of Tibetan plateau and phenotypic characterization of a dominant uncultured methanogen cluster ZC-I. *Environ Microbiol* **10:** 1850-1860.

Zhang G, Zhang J, Ren J, Li J, Liu S (2008b). Distributions and sea-to-air fluxes of methane and nitrous oxide in the North East China Sea in summer. *Mar Chem***110:** 42-55.

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

Zhang K, Shi Y, Cui X, Yue P, Li K, Liu X *et al* (2019). Salinity is a key determinant for soil microbial communities in a desert ecosystem. *mSystems* **4:** e00225-00218.

Zheng H, Wu H (2010). Gene-centric association analysis for the correlation between the guanine-cytosine content levels and temperature range conditions of prokaryotic species. *BMC Bioinformatics* **11:** S7.

Zheng Y, Zhang LM, Zheng YM, Di HJ, He JZ (2008). Abundance and community composition of methanotrophs in a Chinese paddy soil under long-term fertilization practices. *J Soil Sediment* **8:** 406-414.

Zheng Y, Liu XZ, Zhang LM, Zhou ZF, He JZ (2010). Do land utilization patterns affect methanotrophic communities in a Chinese upland red soil? *J Environ Sci-China* **22:** 1936-1943.

Zheng Y, Yang W, Sun X, Wang SP, Rui YC, Luo CY *et al* (2012). Methanotrophic community structure and activity under warming and grazing of alpine meadow on the Tibetan Plateau. *Appl Microbiol Biot* **93:** 2193-2203.

Zhou X, Zhang Z, Tian L, Li X, Tian C (2017). Microbial communities in peatlands along a chronosequence on the Sanjiang Plain, China. *Sci Rep* **7:** 9567.

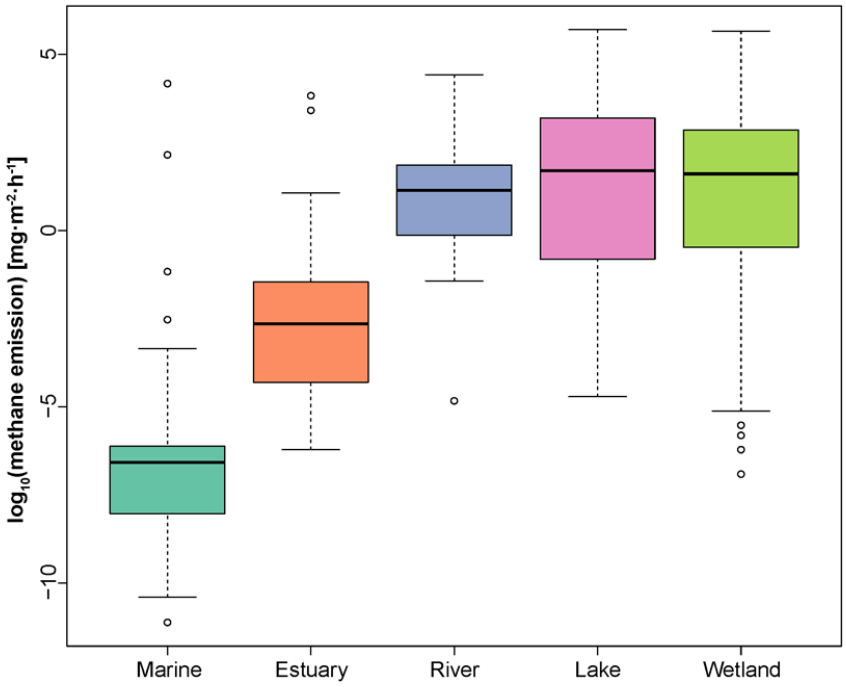
Zhou Z, Chen J, Cao H, Han P, Gu JD (2014). Analysis of methane-producing and metabolizing archaeal and bacterial communities in sediments of the northern South China Sea and coastal Mai Po Nature Reserve revealed by PCR amplification of *mcrA* and *pmoA* genes. *Front Microbiol* **5:** 789.

Zinder SH (1993). Physiological Ecology Of Methanogens. In: Ferry JG (ed). *Methanogenesis*. Springer: Boston, MA. pp 128-206.

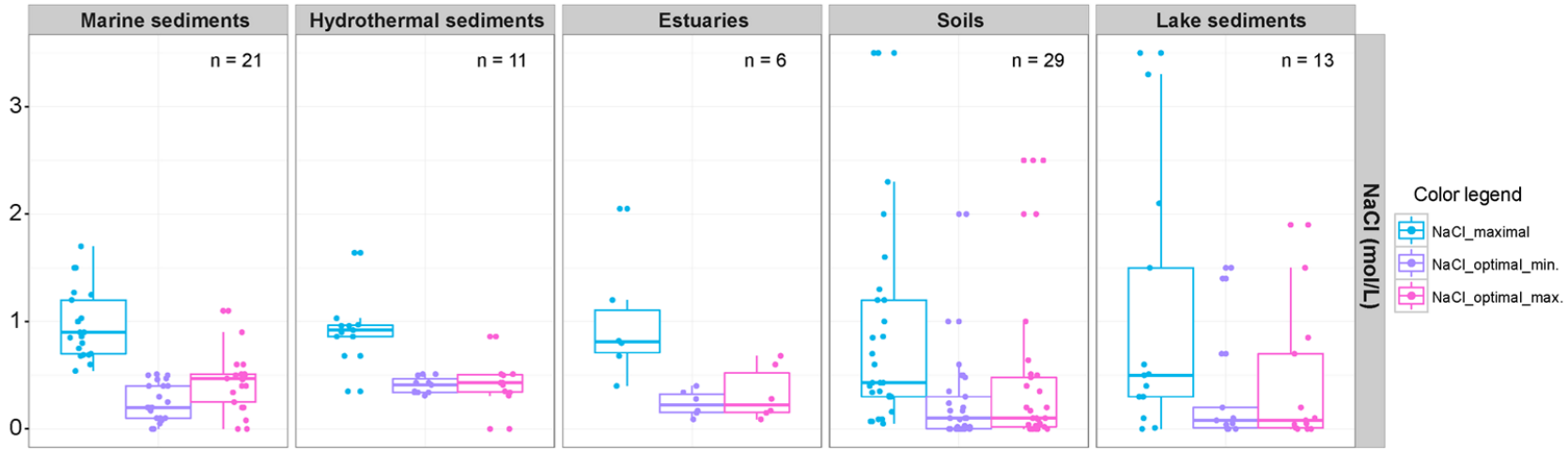
Zinger L, Amaral-Zettler LA, Fuhrman JA, Horner-Devine MC, Huse SM, Welch DB *et al* (2011). Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. *Plos One* **6:** e24570.

Appendix

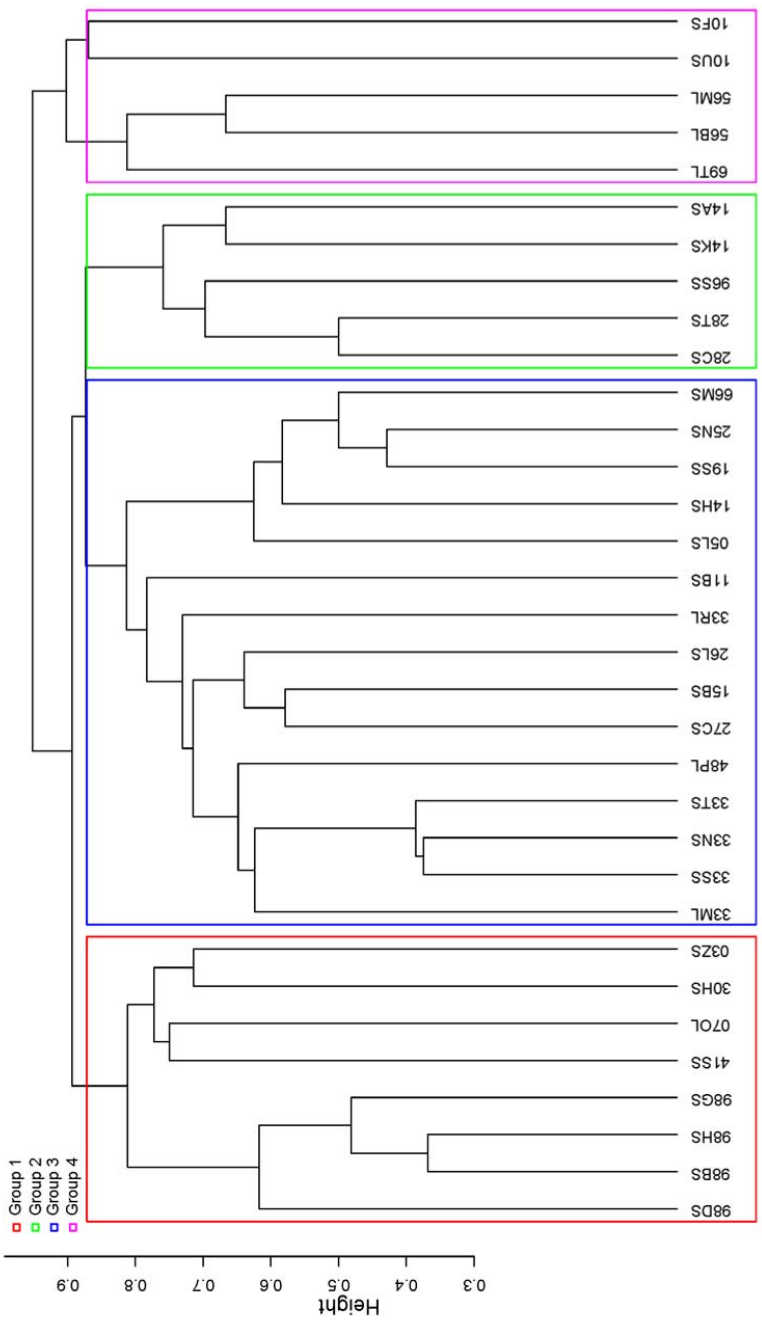
A Supplementary materials for Manuscript I



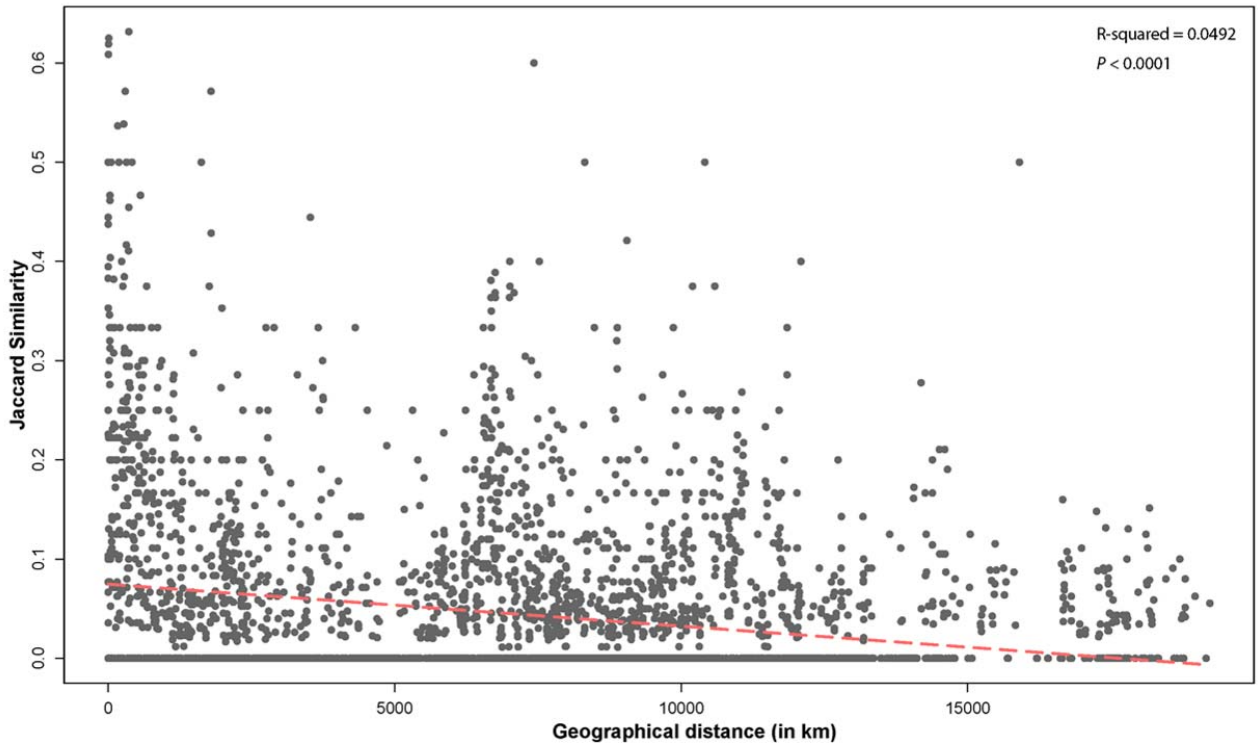
Supplementary Figure A.1 Methane emission rates from various natural ecosystems. The wetlands and lakes possessed the highest methane emission rate, while the oceans had the lowest. Estuary environments showed the intermediate emission rate. The original data for unmanaged natural environments are mainly according to (Ortiz-Llorente and Alvarez-Cobelas 2012). In addition, we hereby included data some estuary sites published in other studies (Abril and Iversen 2002, Angelis and Scranton 1993, Burgos et al 2015, Bussmann 2013, Mackelprang et al 2011, Middelburg et al 2002, Shakhova and Semiletov 2007, Zhang et al 2008b). These research sites were classified into 5 ecosystems, namely, marine, estuary, river, lake and wetland based on the original sites description. The plot used the $\log_{10}$-transformed data.



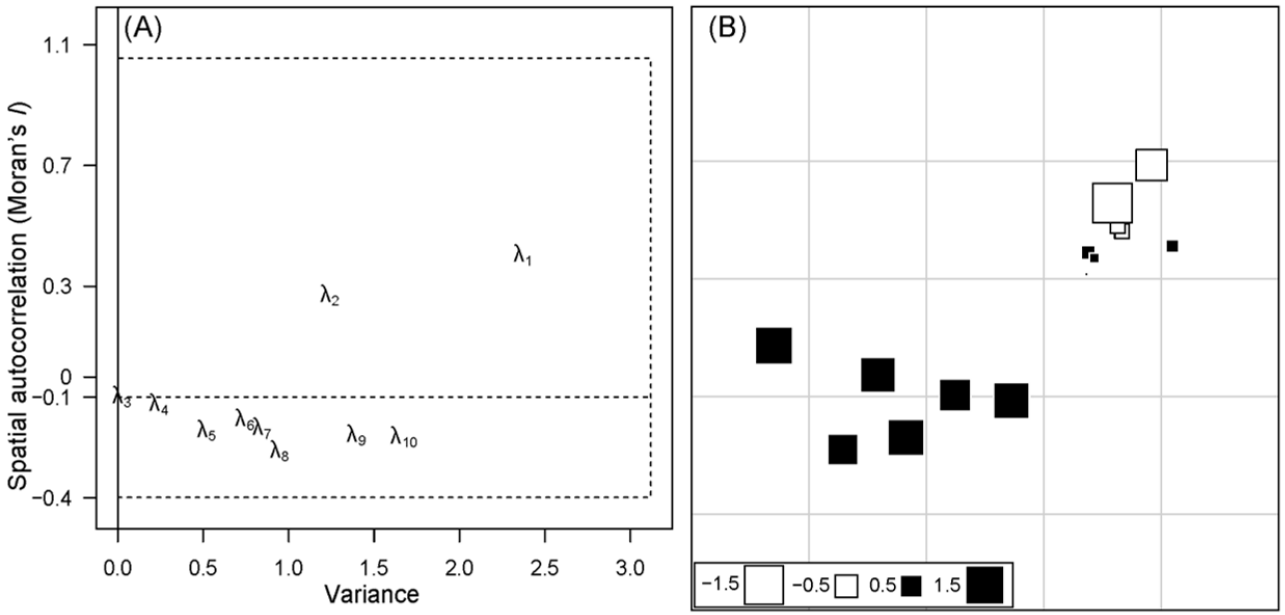
Supplementary Figure A.2 Box plot showing the quantile summary of NaCl tolerance of 80 methanogenic isolates from various habitats. These isolates were categorized into 5 environments, namely, marine sediments, hydrothermal sediments, estuaries, soils and lake sediments; "n" denotes the number of isolates in each environment. For NaCl, the maximal tolerance, minimum and maximum optima are summarized. The original data is available from http://metanogen.biotech.uni.wroc.pl/ (Jabłoński et al 2015).



Supplementary Figure A.3 The clustering dendrogram based on a matrix of Jaccard distance among 33 nonsaline soil and lake sediment sites. The UPGMA method was used for the cluster analysis. According to the cluster results, the 33 sites are divided into 4 groups, which are indicated by different colored rectangles in the dendrogram.



Supplementary Figure A.4 Scatterplot of the incidence-based Jaccard similarity between methanogenic communities in natural environments and their global geographic distance. Each point corresponds to one pairwise comparison of 4371 points from 94 sampling sites. The red-dashed line shows a fitted linear linear model, with correlation coefficient given in the top right of the plot.



Supplementary Figure A.5 Spatial PCA on 16 European soil and lake sediment samples. Subplot (A) represents the spatial and variance components of the eigenvalues (denoted as λ_1, λ_2 … λ_{10}) according to their variance and Moran's I values. λ_1 is the largest eigenvalue in terms of variance and spatial autocorrelation and the first spatial pattern, associated to λ_1, was retained. We mapped the first principle component (PC) associated to λ_1 onto the geographical space (B) by the 's.value' function in ade4 package (Chessel et al 2004). It uses black and white squares of variable size for positive and negative values, respectively. The first PC shows a spatial structure that the samples are divided into two clusters, one in the north and one in the middle of Europe.

Supplementary Table A.1 Locations, representative sequences amounts, types of habitats and salinity of the 94 libraries

Index	Location	Lon	Lat	Seq. Num.	Habitats	Salinity	Elv. (m)	MAAT	MAP	pH	References
03ZS	Zoige wetland, Qinghai-Tibet Plateau	102.87	33.93	23	soil	nonsaline	3485	0.8	720	7.3	Zhang et al 2008a
05LS	Lakkasuo, central Finland	24.30	61.78	18	soil	nonsaline	159	3	650	4.8	Juottonen et al 2005
07OL	Oude Waal, the Netherlands	5.88	51.85	15	lake sediment	nonsaline	11	9.7	744	7.3	Kemnitz et al 2004
10FS	The Florida Everglades, USA	-80.36	26.35	28	soil	nonsaline	6	23	1168	7.5	Castro et al 2004
10US	The Florida Everglades, USA	-80.41	26.29	17	soil	nonsaline	4	23	1168	7.5	Castro et al 2004
11BS	Bear Meadows Bog, USA	-77.76	40.65	92	soil	nonsaline	352	10	2130	4.4	Steinberg and Regan 2008
14AS	Asusuo, Finland	23.63	60.43	9	soil	nonsaline	90	6.1	710	4.6	Juottonen et al 2012
14HS	Hirsikangas, Finland	26.67	64.07	10	soil	nonsaline	180	2.8	630	5.4	Juottonen et al 2012
14KS	Kallioneva, Finland	23.80	62.27	9	soil	nonsaline	143	3.5	630	5.0	Juottonen et al 2012
15BS	Bibai, Hokkaido, Japan	141.80	43.32	15	soil	nonsaline	16	6.6	1124	4.3	Narihiro et al 2011
19SS	Salmisuo mire complex, Finland	62.79	30.93	21	soil	nonsaline	153	2	600	4.3	Galand et al 2002
23JE	Jiulong River estuary, China	117.94	24.41	47	estuary	mixed	2	NA	NA	NA	Li et al 2012
25NS	northern Finland	26.30	64.50	25	soil	nonsaline	108	1.5	505	3.9	Juottonen et al 2006
26LS	Lakkasuo mire complex, Finland	24.32	61.80	15	soil	nonsaline	155	3	650	4.6	Galand et al 2005
27CS	Canton of Bern, Switzerland	8.26	46.53	30	soil	nonsaline	2084	3.3	1800	4.7	Franchini and Zeyer 2012
28CS	Bøttemyra wetland, Norway	29.20	69.69	147	soil	nonsaline	85	-0.6	435	4.3	Liebner et al 2015

28TS	Bøttemyra wetland, Norway	29.20	69.68	172	soil	nonsaline	84	-0.6	435	4.1	Liebner et al 2015
30HS	Herschel Island, Canadian Arctic	-138.96	69.58	79	soil	nonsaline	27	-11.7	155	5.4	Barbier et al 2012
31ME	Marennes-Oleron Bay, France	-1.67	45.95	36	estuary	mixed	-34	NA	NA	NA	Roussel et al 2009
33ML	Mary lake, USA	-89.90	46.25	158	lake sediment	nonsaline	499	4.6	814	5.6	Youngblut et al 2014
33NS	North sparkling bog, USA	-89.70	46.10	76	soil	nonsaline	496	4.6	814	4.6	Youngblut et al 2014
33RL	Rose lake, USA	-89.90	46.25	81	lake sediment	nonsaline	499	4.6	814	6.5	Youngblut et al 2014
33SS	South sparkling bog, USA	-89.70	46.00	130	soil	nonsaline	496	4.6	814	4.3	Youngblut et al 2014
33TS	Trout bog, USA	-89.69	46.04	75	soil	nonsaline	500	4.6	814	4.4	Youngblut et al 2014
34PL	Priest Pot, UK	-2.98	54.35	17	lake sediment	nonsaline	250	9.4	1521	NA	Earl et al 2003
40BE	Beaulieu Estuary, UK	-1.45	50.82	26	estuary	mixed	22	NA	NA	NA	Banning et al 2005
41SS	Sitka stream, Czech Republic	17.25	49.65	25	soil	nonsaline	225	5.61	550	6.8	Buriánková et al 2013
48PL	Lake Pavin, France	2.89	45.50	69	lake sediment	nonsaline	1265	6.5	1650	7.8	Biderre-Petit et al 2011
50PE	Dongtan, China	121.92	31.52	47	estuary	mixed	2	NA	NA	NA	Zeleke et al 2013b
50SE	Dongtan, China	121.92	31.52	58	estuary	mixed	2	NA	NA	NA	Zeleke et al 2013b
50TE	Dongtan, China	121.92	31.52	38	estuary	mixed	2	NA	NA	NA	Zeleke et al 2013b
52YE	Yangtze river estuary, China	121.08	31.05	88	estuary	mixed	6	NA	NA	NA	Zeleke et al 2013a
56BL	Lake Batata, Amazon River	-56.25	-1.42	44	lake sediment	nonsaline	31	26.5	1888	6.9	Conrad et al 2010
56ML	Lake Mussura, Amazon River	-56.43	-1.58	44	lake sediment	nonsaline	81	26.5	1888	7.2	Conrad et al 2010
61LL	Lonar lake, India	76.52	19.98	9	lake sediment	saline	541	26.6	729	9.9	Antony et al 2012
62PE	Pearl river estuary, China	113.64	22.46	17	estuary	mixed	7	NA	NA	NA	Jiang et al 2011

Supplementary Table A.1 Locations, representative sequences amounts, types of habitats and salinity of the 94 libraries

Index	Location	Lon	Lat	Seq. Num.	Habitats	Salinity	Elv. (m)	MAAT	MAP	pH	References
03ZS	Zoige wetland, Qinghai-Tibet Plateau	102.87	33.93	23	soil	nonsaline	3485	0.8	720	7.3	Zhang et al 2008a
05LS	Lakkasuo, central Finland	24.30	61.78	18	soil	nonsaline	159	3	650	4.8	Juottonen et al 2005
07OL	Oude Waal, the Netherlands	5.88	51.85	15	lake sediment	nonsaline	11	9.7	744	7.3	Kemnitz et al 2004
10FS	The Florida Everglades, USA	-80.36	26.35	28	soil	nonsaline	6	23	1168	7.5	Castro et al 2004
10US	The Florida Everglades, USA	-80.41	26.29	17	soil	nonsaline	4	23	1168	7.5	Castro et al 2004
11BS	Bear Meadows Bog, USA	-77.76	40.65	92	soil	nonsaline	352	10	2130	4.4	Steinberg and Regan 2008
14AS	Asusuo, Finland	23.63	60.43	9	soil	nonsaline	90	6.1	710	4.6	Juottonen et al 2012
14HS	Hirsikangas, Finland	26.67	64.07	10	soil	nonsaline	180	2.8	630	5.4	Juottonen et al 2012
14KS	Kallioneva, Finland	23.80	62.27	9	soil	nonsaline	143	3.5	630	5.0	Juottonen et al 2012
15BS	Bibai, Hokkaido, Japan	141.80	43.32	15	soil	nonsaline	16	6.6	1124	4.3	Narihiro et al 2011
19SS	Salmisuo mire complex, Finland	62.79	30.93	21	soil	nonsaline	153	2	600	4.3	Galand et al 2002
23JE	Jiulong River estuary, China	117.94	24.41	47	estuary	mixed	2	NA	NA	NA	Li et al 2012
25NS	northern Finland	26.30	64.50	25	soil	nonsaline	108	1.5	505	3.9	Juottonen et al 2006
26LS	Lakkasuo mire complex, Finland	24.32	61.80	15	soil	nonsaline	155	3	650	4.6	Galand et al 2005
27CS	Canton of Bern, Switzerland	8.26	46.53	30	soil	nonsaline	2084	3.3	1800	4.7	Franchini and Zeyer 2012
28CS	Bøttemyra wetland, Norway	29.20	69.69	147	soil	nonsaline	85	-0.6	435	4.3	Liebner et al 2015

28TS	Bøttemyra wetland, Norway	29.20	69.68	172	soil	nonsaline	84	-0.6	435	4.1	Liebner et al 2015
30HS	Herschel Island, Canadian Arctic	-138.96	69.58	79	soil	nonsaline	27	-11.7	155	5.4	Barbier et al 2012
31ME	Marennes-Oleron Bay, France	-1.67	45.95	36	estuary	mixed	-34	NA	NA	NA	Roussel et al 2009
33ML	Mary lake, USA	-89.90	46.25	158	lake sediment	nonsaline	499	4.6	814	5.6	Youngblut et al 2014
33NS	North sparkling bog, USA	-89.70	46.10	76	soil	nonsaline	496	4.6	814	4.6	Youngblut et al 2014
33RL	Rose lake, USA	-89.90	46.25	81	lake sediment	nonsaline	499	4.6	814	6.5	Youngblut et al 2014
33SS	South sparkling bog, USA	-89.70	46.00	130	soil	nonsaline	496	4.6	814	4.3	Youngblut et al 2014
33TS	Trout bog, USA	-89.69	46.04	75	soil	nonsaline	500	4.6	814	4.4	Youngblut et al 2014
34PL	Priest Pot, UK	-2.98	54.35	17	lake sediment	nonsaline	250	9.4	1521	NA	Earl et al 2003
40BE	Beaulieu Estuary, UK	-1.45	50.82	26	estuary	mixed	22	NA	NA	NA	Banning et al 2005
41SS	Sitka stream, Czech Republic	17.25	49.65	25	soil	nonsaline	225	5.61	550	6.8	Buriánková et al 2013
48PL	Lake Pavin, France	2.89	45.50	69	lake sediment	nonsaline	1265	6.5	1650	7.8	Biderre-Petit et al 2011
50PE	Dongtan, China	121.92	31.52	47	estuary	mixed	2	NA	NA	NA	Zeleke et al 2013b
50SE	Dongtan, China	121.92	31.52	58	estuary	mixed	2	NA	NA	NA	Zeleke et al 2013b
50TE	Dongtan, China	121.92	31.52	38	estuary	mixed	2	NA	NA	NA	Zeleke et al 2013b
52YE	Yangtze river estuary, China	121.08	31.05	88	estuary	mixed	6	NA	NA	NA	Zeleke et al 2013a
56BL	Lake Batata, Amazon River	-56.25	-1.42	44	lake sediment	nonsaline	31	26.5	1888	6.9	Conrad et al 2010
56ML	Lake Mussura, Amazon River	-56.43	-1.58	44	lake sediment	nonsaline	81	26.5	1888	7.2	Conrad et al 2010
61LL	Lonar lake, India	76.52	19.98	9	lake sediment	saline	541	26.6	729	9.9	Antony et al 2012
62PE	Pearl river estuary, China	113.64	22.46	17	estuary	mixed	7	NA	NA	NA	Jiang et al 2011

63MV	eastern Mediterranean Sea	30.56	35.43	9	mud volcano	saline	-1885	NA	NA	NA	Kormas et al 2008
64BL	Bitter lakes system, south-eastern Siberia	79.90	51.67	9	lake sediment	saline	164	0	300	10.2	Unpublished
64CL	Cock lake, south-eastern Siberia	79.15	52.10	15	lake sediment	saline	156	0	300	10.3	Unpublished
64TL	Tanatar lakes system, south-eastern Siberia	79.78	51.65	8	lake sediment	saline	157	0	300	10.1	Unpublished
65KM	Kuroshima Knoll, Japan	124.20	24.13	6	marine sediment	saline	-1052	NA	NA	NA	Inagaki et al 2004
66MS	near river Plesna, Czech	12.45	50.15	62	soil	nonsaline	468	7.5	598	5.3	Beulig et al 2015
68TV	Lei-Gong-Hou mud volcanoes, Taiwan	121.21	22.98	9	mud volcano	Not defined	364	NA	NA	NA	Wang et al 2014
69TL	Tucurui dam, Brazil	-49.67	-3.75	112	lake sediment	nonsaline	43	24	2050	6.3	Santana et al 2012
70TL	Tirez lagoon, La Mancha	-3.35	39.55	11	lake sediment	saline	660	14.8	400	7.2	Montoya et al 2011
71NM	Nankai Trough, the Pacific Ocean	135.03	32.24	18	marine sediment	saline	-4785	NA	NA	NA	Newberry et al 2004
72TL	lake Therm-Organ, Hungary	20.61	46.90	4	lake sediment	saline	80	10.7	530	9.0	Porsch et al 2015
73ME	Min River estuary, China	119.57	26.01	37	estuary	mixed	224	NA	NA	NA	She and Tong 2012
743H	Guaymas Basin	-111.41	27.01	7	hydrothermal sediment	saline	-1963	NA	NA	NA	Biddle et al 2012
746H	Guaymas Basin	-111.41	27.01	17	hydrothermal sediment	saline	-1962	NA	NA	NA	Biddle et al 2012
749H	Guaymas Basin	-111.41	27.01	11	hydrothermal sediment	saline	-1962	NA	NA	NA	Biddle et al 2012
75AH	Axial Volcano, northeastern Pacific Ocean	-129.98	45.93	445	hydrothermal sediment	saline	-1958	NA	NA	NA	Ver Eecke et al 2012

75EH	Endeavour Segment, NE Pacific Ocean	-129.10	47.95	87	hydrothermal sediment	saline	-2372	NA	NA	NA	Ver Eecke et al 2012
76CE	Colne estuary, UK	0.96	51.92	17	estuary	saline	34	NA	NA	NA	Oakley et al 2012
77RH	Rainbow, Atlantic	-33.90	36.24	4	hydrothermal sediment	saline	-2673	NA	NA	NA	Roussel et al 2011
78AV	Eastern Mediterranean Sea	30.27	35.33	7	mud volcano	saline	-2139	NA	NA	NA	Lazar et al 2012
79CE	estuary of the Canane´ia, Brazil	-47.92	-25.07	145	estuary	mixed	26	NA	NA	NA	Taketani et al 2010
801E	Mai Po Natural Reverse	114.03	22.50	211	estuary	mixed	5	NA	NA	NA	Zhou et al 2014
802E	Mai Po Natural Reverse	114.03	22.50	201	estuary	mixed	7	NA	NA	NA	Zhou et al 2014
803E	Mai Po Natural Reverse	114.05	22.49	48	estuary	mixed	14	NA	NA	NA	Zhou et al 2014
80AM	South China Sea	119.98	21.52	47	marine sediment	saline	-3015	NA	NA	NA	Zhou et al 2014
80BM	South China Sea	120.00	18.48	48	marine sediment	saline	-2313	NA	NA	NA	Zhou et al 2014
80CM	South China Sea	115.22	19.92	47	marine sediment	saline	-1189	NA	NA	NA	Zhou et al 2014
80DM	South China Sea	119.28	22.67	46	marine sediment	saline	-91	NA	NA	NA	Zhou et al 2014
80EM	South China Sea	115.22	19.63	42	marine sediment	saline	-1970	NA	NA	NA	Zhou et al 2014
80FM	South China Sea	114.73	20.25	45	marine sediment	saline	-168	NA	NA	NA	Zhou et al 2014
80GM	South China Sea	114.25	20.73	46	marine sediment	saline	-90	NA	NA	NA	Zhou et al 2014
80HM	South China Sea	114.00	21.00	47	marine sediment	saline	-70	NA	NA	NA	Zhou et al 2014
80IM	South China Sea	113.75	21.33	50	marine sediment	saline	-51	NA	NA	NA	Zhou et al 2014
80JM	South China Sea	113.50	21.48	53	marine sediment	saline	-40	NA	NA	NA	Zhou et al 2014

80KM	South China Sea	111.27	19.50	50	marine sediment	saline	-90	NA	NA	NA	Zhou et al 2014
80LM	South China Sea	116.80	22.85	46	marine sediment	saline	-33	NA	NA	NA	Zhou et al 2014
81MV	Meknes MV, Gulf of Cadiz, Spain	7.07	34.99	8	mud volcano	saline	998	NA	NA	NA	Sas 2009
82PM	Peru margin	-78.00	-11.00	8	marine sediment	saline	-183	NA	NA	NA	Parkes et al 2005
83FM	Florida Escarpment, Gulf of Mexico	-84.92	26.03	16	marine sediment	saline	-2482	NA	NA	NA	Reed et al 2009
83RH	Rainbow hydrothermal vent, Mid-Atlantic Ridge	-33.90	36.23	8	hydrothermal sediment	saline	-2732	NA	NA	NA	Reed et al 2009
84EH	Everest Mound area, Guaymas vent field	-111.41	27.01	13	hydrothermal sediment	saline	-1964	NA	NA	NA	Dhillon et al 2005
85SM	Shimokita Peninsula, Japan	142.20	41.18	34	marine sediment	saline	-1179	NA	NA	NA	Nunoura et al 2016
86CM	Cascadia Margin, Canada	-126.87	48.70	20	marine sediment	saline	-1310	NA	NA	NA	Yoshioka et al 2010
87SM	Sonora Margin cold seeps, Guaymas Basin	-111.48	27.60	13	marine sediment	saline	-1669	NA	NA	NA	Vigneron et al 2014
88GM	G11 Nyegga pockmark, Norwegian Sea	5.29	64.67	52	marine sediment	saline	-752	NA	NA	NA	Lazar et al 2011a
89NV	Napoli mud volcano, Eastern Mediterranean Sea	24.69	33.73	108	mud volcano	saline	-2035	NA	NA	NA	Lazar et al 2011b
90SM	Santa Barbara Basin, California	-119.99	34.23	9	marine sediment	saline	-558	NA	NA	NA	Harrison et al 2009
91GM	Green Canyon 205, Gulf of Mexico	-90.53	27.72	8	marine sediment	saline	-877	NA	NA	NA	Lloyd et al 2006
92KV	Nankai Trough	136.56	33.60	10	mud volcano	saline	-2068	NA	NA	NA	Miyazaki et al 2009

94CL	Cornwallis Island, Canada	-94.90	74.76	6	lake sediment	nonsaline	59	-16.5	131	8.0	Stoeva et al 2014
94CS	Cornwallis Island, Canada	-94.08	74.76	10	soil	nonsaline	196	-16.5	131	8.1	Stoeva et al 2014
95CS	Chongxi wetland, Shanghai, China	121.21	31.73	28	soil	nonsaline	1	15.3	1049	NA	Unpublished
96SS	Suonukkasuo, Finland	25.85	66.47	24	soil	nonsaline	118	0.9	577	4.3	Yrjälä et al 2011
97LH	Lost city hydrothermal field	-42.12	30.12	7	hydrothermal sediment	saline	-3849	NA	NA	NA	Kelley et al 2005
98BS	Haibei, Qinghai-Tibet Plateau, China	101.32	37.62	46	soil	nonsaline	3202	-1.7	500	6.5	Yang et al 2017
98DS	Donggi Cona lake, Qinghai-Tibet Plateau, China	98.50	35.35	69	soil	nonsaline	4208	-4.1	300	8.2	Yang et al 2017
98GS	Gande, Qinghai-Tibet Plateau, China	100.05	34.02	49	soil	nonsaline	4247	-2.2	550	6.0	Yang et al 2017
98HS	Huashixia, Qinghai-Tibet Plateau, China	98.78	35.10	43	soil	nonsaline	4407	-4.1	304	8.2	Yang et al 2017

Note: Lon, Longitude; Lat: Latitude; Seq. Num.: Sequence numbers; Elv.: Elevation; MAAT: Mean Annual Air Temperature; MAP: Mean Annual Precipitation; NA: not available.

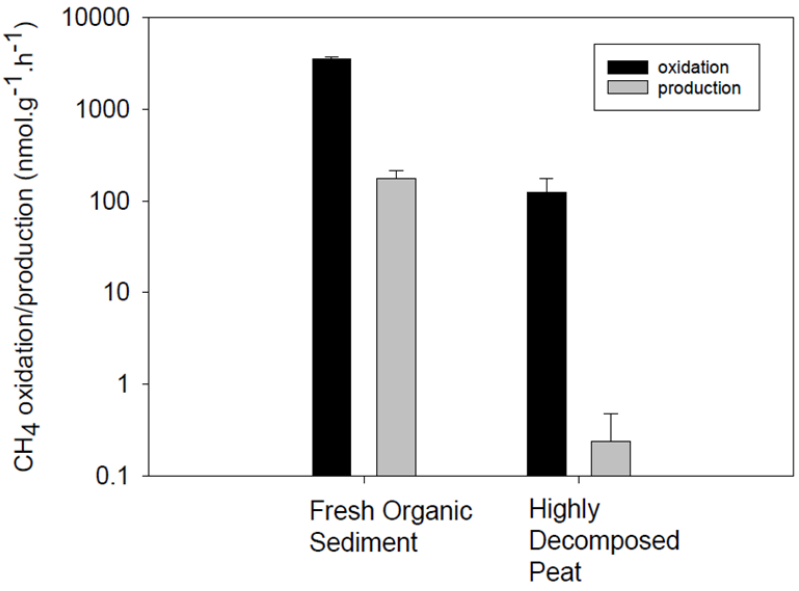
Supplementary Table A.2 Number of samples, sequences and OTUs of the 6 habitats (marine sediment, hydrothermal sediment, mud volcano, estuary, lake sediment and soil) defined in this study.

Habitat	Number of samples	Total sequences	Number of OTUs
Estuary	14	1016	144
Soil	29	1362	123
Lake sediment	14	587	75
Marine sediment	22	751	60
Mud volcano	6	151	12
Hydrothermal sediment	9	599	32

Supplementary Table A.3 Significance of Wilcoxon rank sum test of the richness (Chao2 indices) differences at OTU level between the six natural environment types. Significant differences ($P < 0.05$) are marked with asterisk. The null hypothesis is that the diversity of methanogenic communities between habitats was identical; the alternative hypothesis "greater" was used to test if the diversity in a habitat (in row) is significantly higher than in another habitat (in column). Mud volcano has only one observation is thus excluded in the statistic test.

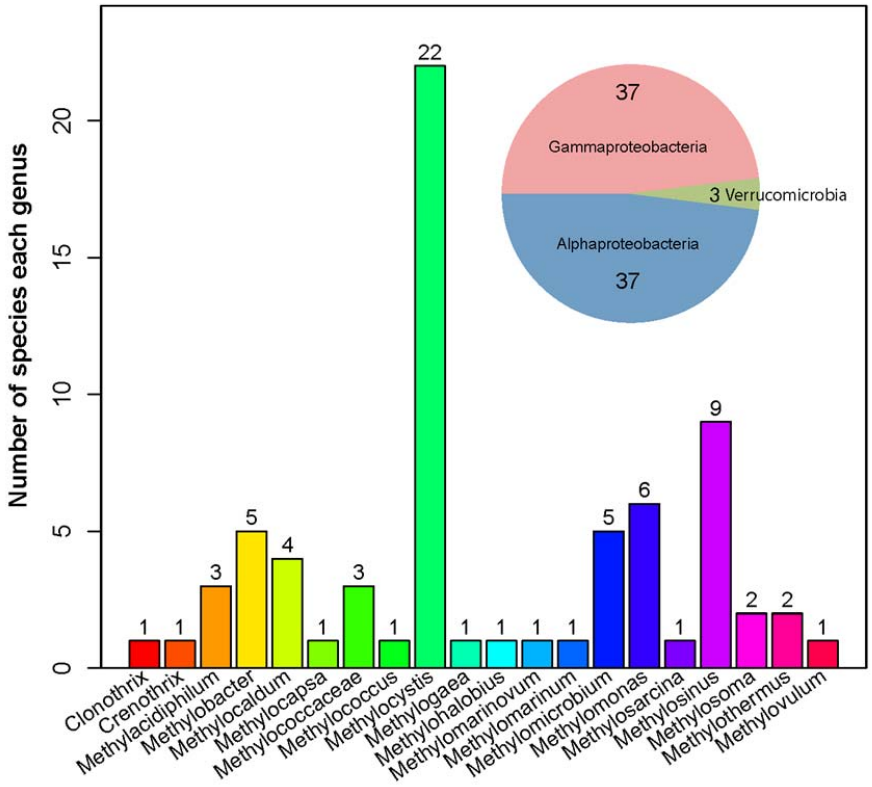
	Soils	Lake sediments	Marine sediments	Hydrothermal sediments
Estuaries	0.0324*	0.0115*	0.0020*	0.0161*
Soils		0.2071	0.0011*	0.0349*
Lake sediments			0.0424*	0.0500*
Marine sediments				0.4401

B Supplementary Materials of Manuscript II

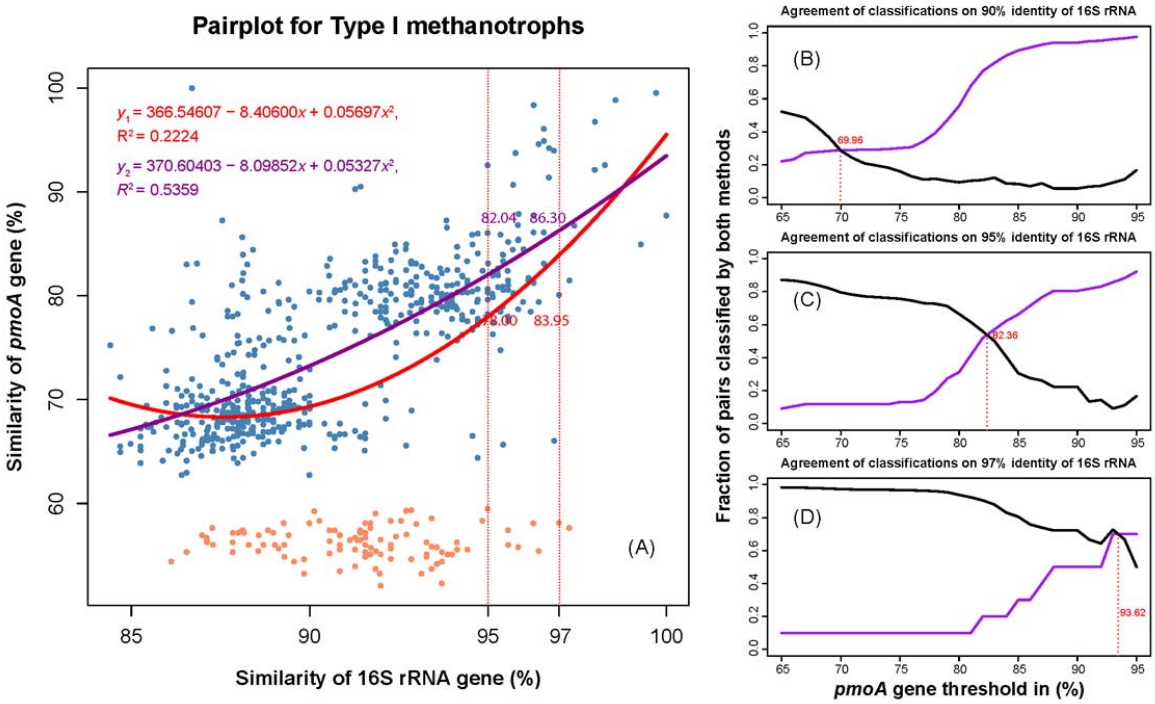


Supplementary Figure A.6 Incubation data from Zarnekow, a freshwater minerotrophic fen in Northeastern Germany. Rates of methane production (n=3) and methane oxidation (n=3) are shown for both fresh (surficial) organic sediment and the bulk peat.

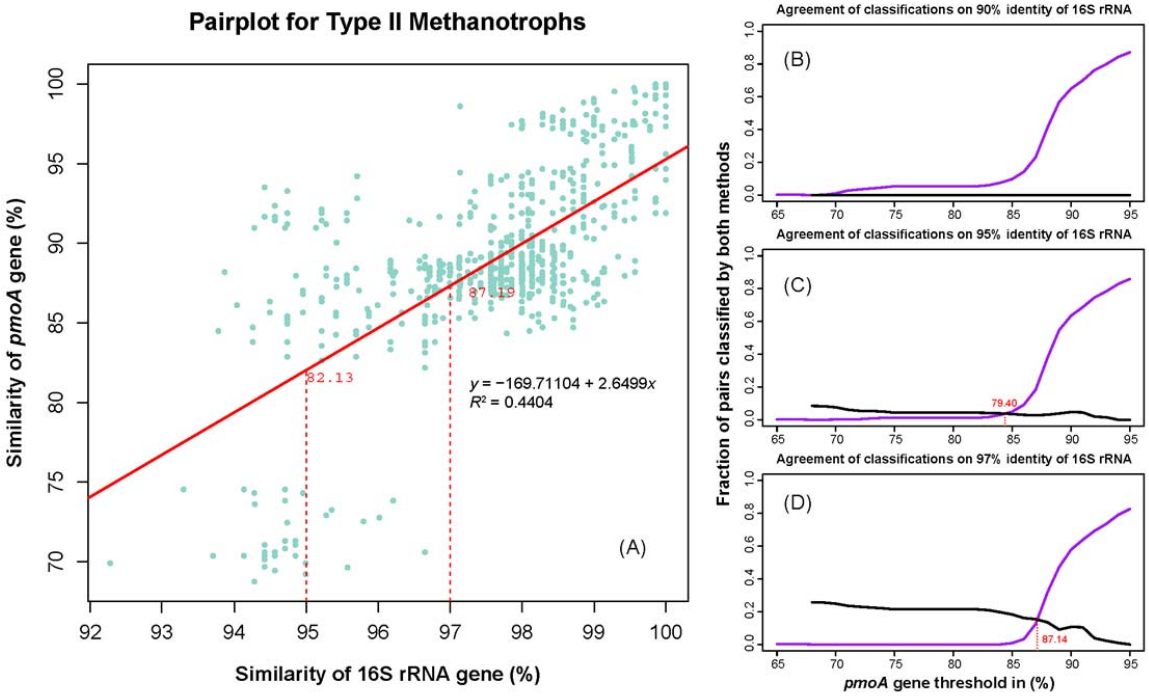
C Supplementary Materials of Manuscript III



Supplementary Figure A.7 Taxonomic distribution of species used in this study.



Supplementary Figure A.8 Pairwise plot for the type I (Gammaproteobacteria) methanotrophs. The dot in light red shows the outliers caused by *Crenothrix* and *Methylococcaceae bacterium* M200. The blue dashed and red solid lines represent the regression including and excluding outliers, respectively. The right column shows the fraction of pairs classified by both methods at fixed cutoff-values for the 16S rRNA gene at 90%, 95%, and 97% sequence identity, in the way similar to Figure 4.2.



Supplementary Figure A.9 Pairwise plot for the type II (Alphaproteobacteria) methanotrophs. The three small plots in the right column shows the fraction of pairs classified by both methods at fixed cutoff-values for the 16S rRNA gene at 90%, 95%, and 97% sequence identity.

Supplementary Table A.4 Summary of 77 species used in this study

Species	Acc_*pmoA*	Acc_16S	gene type	Taxonomic type
Methylomicrobium kenyense	JN687579	AJ132384	*pmoA*	Gammaproteobacteria
Methylocaldum sp. BFH1	GQ130270	GQ130271	*pmoA*	Gammaproteobacteria
Methylocystis heyeri	AM283546	AM285681	*pmoA*	Alphaproteobacteria
Methylohalobius crimeensis	AJ581836	NR_042198	*pmoA*	Gammaproteobacteria
Methylocystis sp. KS7	AJ459034	AJ458498	*pmoA*	Alphaproteobacteria
Methylosoma difficile	DQ119047	NR_043562	*pmoA*	Gammaproteobacteria
Methylosinus sp. LW4	AY007282	AY007293	*pmoA*	Alphaproteobacteria
Methylosinus sp. W3-6	AB371599	AB371594	*pmoA*	Alphaproteobacteria
Methylocystis sp. M162	JN036527	JN036511	*pmoA*	Alphaproteobacteria
Methylococcaceae bacterium M200	HM564019	HM564015	*pxmA*	Gammaproteobacteria
Clonothrix fusca	DQ984192	DQ984190	*pmoA*	Gammaproteobacteria
Methylocapsa acidiphila	CT005238	NR_028923	*pmoA*	Alphaproteobacteria
Methylocystis hirsuta	DQ364434	NR_043754	*pmoA*	Alphaproteobacteria
Methylomicrobium buryatense	AF307139	NR_025136	*pmoA*	Gammaproteobacteria
Methylomicrobium album	EU722431	X72777	*pmoA*	Gammaproteobacteria
Methylomonas sp. M5	HM564020	HM564016	*pxmA*	Gammaproteobacteria
Methylocystis sp. M212	JN036528	JN036516	*pmoA*	Alphaproteobacteria
Methylomonas koyamae	AB538965	AB538964	*pmoA*	Gammaproteobacteria
Methylocystis parvus	AF533665	AF150805	*pmoA*	Alphaproteobacteria
Methylosoma sp. TFB	GQ130273	GQ130272	*pmoA*	Gammaproteobacteria
Methylocaldum sp. 0510-P-2	EU275142	EU275144	*pmoA*	Gammaproteobacteria
Methylosinus sp. R62	AB371597	AB371593	*pmoA*	Alphaproteobacteria
Methylomonas sp. MG30	HE801217	NR_108887	*pmoA*	Gammaproteobacteria
Methylocystis sp. 5FB2	AJ868407	AJ868420	*pmoA*	Alphaproteobacteria
methanotroph FL20	AF182471	AF183828	*pmoA*	Alphaproteobacteria
Methylocaldum sp. 05J-I-7	EU275141	EU275146	*pmoA*	Gammaproteobacteria

thermophilic methanotroph HB	U89302	TMU89299	*pmoA*	Gammaproteobacteria
Methylocystis sp. 51	AJ459004	AJ458475	*pmoA*	Alphaproteobacteria
Methylocystis sp. IMET 10489	AJ458999	AJ458472	*pmoA*	Alphaproteobacteria
Methylosinus sp. M1	AB371596	AB371591	*pmoA*	Alphaproteobacteria
Methylomicrobium pelagicum	U31652	NR_044848.2	*pmoA*	Gammaproteobacteria
Methylosinus sporium	DQ119048	EF619620	*pmoA*	Alphaproteobacteria
Methylomonas methanica	EU722433	NR_074627	*pmoA*	Gammaproteobacteria
Methylacidiphilum infernorum V4	EU223859	NR_074583.1	*pmoA*	Verrucomicrobia
Methylocystis sp. m261	DQ852354	DQ852351	*pmoA*	Alphaproteobacteria
methanotroph M5	AF182477	AF183834	*pmoA*	Alphaproteobacteria
Methylocystis sp. B3	DQ496238	DQ496232	*pmoA*	Alphaproteobacteria
Methylobacter sp. HG-1	AF495888	AF495887	*pmoA*	Gammaproteobacteria
Methylocystis sp. M175	JN036524	JN036514	*pmoA*	Alphaproteobacteria
Methylocystis sp. S284	HE798547	HE798551.1	*pmoA*	Alphaproteobacteria
Crenothrix polyspora	DQ295903	DQ295890	*pmoA*	Gammaproteobacteria
type II methanotroph AML-A3	AF177327	AF177298	*pmoA*	Alphaproteobacteria
Methylocystis sp. 2-19	AB371595	AB371590	*pmoA*	Alphaproteobacteria
Methylocystis sp. SH31p	AB636308	AB636303	*pmoA*	Alphaproteobacteria
Methylocaldum tepidum	U89304	MTU89297	*pmoA*	Gammaproteobacteria
Methylococcus capsulatus str. Bath	AE017282	NR_074213.1	*pmoA*	Gammaproteobacteria
Methylovulum miyakonense HT12	AB501288	NR_112920.1	*pmoA*	Gammaproteobacteria
methanotroph WI-14	AF182475	AF183832	*pmoA*	Alphaproteobacteria
Methylomonas sp. LW16	AF150797	AF150796	*pmoA*	Gammaproteobacteria
Methylocystis sp. m1511	DQ852352	DQ852349	*pmoA*	Alphaproteobacteria
Methylocystis sp. SS2C	AB636307	AB636302	*pmoA*	Alphaproteobacteria
Methylobacter sp. LW1	AY007285	AF150784	*pmoA*	Gammaproteobacteria
Methylobacter psychrophilus	AY945762	AF152597	*pmoA*	Gammaproteobacteria
Methylobacter sp.	AF016982	AF016981	*pmoA*	Gammaproteobacteria

BB5.1				
Methylococcaceae bacterium OS501	AB636304	AB636299	*pmoA*	Gammaproteobacteria
Methylacidiphilum fumariolicum SolV	CAHT01000087	EF591088	*pmoA*	Verrucomicrobia
Methylomarinum vadi	AB302947	NR_112675	*pmoA*	Gammaproteobacteria
Methylocystis sp. LW5	AF150791	AF150790	*pmoA*	Alphaproteobacteria
Methylosinus trichosporium OB3b	ADVE01000127	NR_044947.1	*pmoA*	Alphaproteobacteria
Methylacidiphilum kamchatkense	FJ462788	EF127896	*pmoA*	Verrucomicrobia
Methylomicrobium alcaliphilum	FO082060	NR_074649	*pmoA*	Gammaproteobacteria
Methylococcaceae bacterium SF-BR	AB453965	AB453959	*pmoA*	Gammaproteobacteria
Methylosinus sp. PW1	AF150803	AF150802	*pmoA*	Alphaproteobacteria
Methylocystis sp. M	U81596	MSU81595	*pmoA*	Alphaproteobacteria
Methylocystis sp. L6	AJ868405	AJ868422	*pmoA*	Alphaproteobacteria
methanotroph M8	AF182478	AF183835	*pmoA*	Alphaproteobacteria
Methylosarcina quisquiliarum	AF177326	NR_025040	*pmoA*	Gammaproteobacteria
Methylogaea oryzae JCM 16910	EU359002	EU672873	*pmoA*	Gammaproteobacteria
Methylocystis sp. IMET 10484	AJ458998	AJ458470	*pmoA*	Alphaproteobacteria
Methylocystis rosea SV97	AJ414657	AJ414656	*pmoA*	Alphaproteobacteria
Methylomarinovum caldicuralii	AB302948	NR_125449	*pmoA*	Gammaproteobacteria
Methylosinus sp. B3R	AB636306	AB636301	*pmoA*	Alphaproteobacteria
Methylosinus sp. LW8	AY007284	AY007294	*pmoA*	Alphaproteobacteria
Methylomonas sp. LC 1	DQ119046	DQ119049	*pmoA*	Gammaproteobacteria
Methylobacter tundripaludum SV96	AJ414658	NR_042107.1	*pmoA*	Gammaproteobacteria
Methylothermus thermalis MYHT	AY829010	AY829009	*pmoA*	Gammaproteobacteria
Methylothermus subterraneus	AB536748	AB536747	*pmoA*	Gammaproteobacteria

D Supplementary Materials of co-author paper

Yang S, **Wen X**, Shi Y, Liebner S, Jin H, Perfumo A (2016). Hydrocarbon degraders establish at the costs of microbial richness, abundance and keystone taxa after crude oil contamination in permafrost environments. *Sci Rep* **6**: 37473.