Diversity of Methane-cycling Microorganisms in Soils and Their Relation to Oxygen

Claudia Knief*

Institute of Crop Science and Resource Conservation – Molecular Biology of the Rhizosphere, University of Bonn, Bonn, Germany.

*Correspondence: knief@uni-bonn.de

https://doi.org/10.21775/cimb.033.023

Abstract

Microorganisms are important players in the global methane cycle. Anaerobic methanogenic archaea are largely responsible for methane production, while aerobic methanotrophic bacteria, as well as anaerobic methanotrophic bacteria and archaea, are involved in methane oxidation. In anoxic wetland soils, methanogens produce methane, while methanotrophs act as a filter and reduce methane emissions. In the predominantly oxic upland soils, aerobic methanotrophs oxidize atmospheric methane. This review gives an overview of the diversity of methanogenic and methanotrophic microorganisms, highlights recent discoveries and provides information concerning their occurrence in soils. Recent findings indicate that the methanogenic and methanotrophic lifestyles are more widespread in microorganisms than previously thought, and that the metabolic versatility of some methane-cycling organisms is broader than known from well-characterized cultivated organisms. It also turned out that the control of methanogenic and methanotrophic bacteria by oxygen is more complex than previously thought. The implications this finding may have for the life of these microorganisms in soils and on soil methane fluxes is discussed.

Introduction

Methane cycling microorganisms are of interest for microbiologists since more than a century. Research on these microorganisms was initially largely driven by the curiosity to understand their particular physiology that leads to the production or consumption of methane. While this interest is still a driver, the importance of methane as greenhouse gas has become another important factor, promoting further research on methanogenic and methanotrophic microorganisms. This leads to a continuously better understanding of their physiology and ecology, and it becomes evident that the processes of microbial methane production and consumption are mediated by more complex functional guilds than initially thought. The improved understanding is not only due to the constantly increasing diversity of methanogenic and methanotrophic microorganisms (e.g. Knief, 2015; Kallistova *et al.*, 2017); additionally, the metabolic versatility of these organisms appears to be much broader than previously thought. This became most evident during the last two decades, based on the study of enrichment cultures and isolates representing novel lineages of methanogens and methanotrophs, several of them with properties that have not been observed before in these organisms (Welte, 2018). The use of new high-throughput

approaches for the analysis of organisms in culture or *in situ,* e.g. by deep metagenomic sequencing and the analysis of reconstructed genome information from individual organisms or near isogenic strains, allows the detection of methanogenic and methanotrophic potential in already known or new microbial taxa (Chistoserdova, 2015). This has resulted in the discovery of methanogenic and methanotrophic pathways in organisms that were not known before to represent methanogens or methanotrophs. Important for methane production or uptake in an ecosystem is not only the presence of methanogenic and methanotrophic organisms, but also their activity. Both presence and activity are largely controlled by diverse abiotic and biotic factors. Recent findings indicate that a strict categorization of the diverse organisms concerning their responses to specific environmental factors may not always be possible. In the present review, this will be exemplified focusing on oxygen dependence of methanogenic and methanotrophic microorganisms. Overall, the aims of this review are:

1 provide an update on the global methane budget and describe the role of soils in global methane cycling,
2 provide an update on the diversity of methanogenic archaea, aerobic methanotrophic bacteria and anaerobic methanotrophic archaea and bacteria,
3 present knowledge about the occurrence of these different groups of methane-cycling organisms in wetland and upland soils,
4 synthesize present knowledge about oxygen as a major environmental factor controlling the occurrence and activity of these groups of microorganisms.

The importance of soils as sources and sinks for atmospheric methane

Methane (CH_4) is the most abundant hydrocarbon in the atmosphere with a current mixing ratio of 1.85 ppmv (Dlugokencky, 2018). This exceeds the preindustrial levels of 0.7 ppmv by a factor of approximately 2.5 (Ciais *et al.*, 2013) and is higher than concentrations recorded in ice cores during the past 800,000 years (Loulergue *et al.*, 2008). From 2007 to 2017, the average yearly increase in atmospheric methane concentration was estimated to be 7 ppb, after emissions had transiently declined at the beginning of the 21st century (Dlugokencky, 2018). The reasons for this increase are under discussion, but a contribution of biogenic emissions, probably due to agricultural activities, appears likely (Saunois *et al.*, 2016b).

Increasing atmospheric methane concentrations are critical, because methane is the most important greenhouse gas after carbon dioxide (CO_2), contributing approximately 20% to global warming (Dlugokencky *et al.*, 2011; Kirschke *et al.*, 2013). This is related to its stronger global-warming potential, which is currently estimated to be 28 times stronger compared with CO_2 (Myhre *et al.*, 2013). Methane has a rather short lifetime of approximately 9 years in the atmosphere (Saunois *et al.*, 2016a), so that effective mitigation strategies could lead to near-term reductions in atmospheric concentrations and could complement CO_2 mitigation strategies (Saunois *et al.*, 2016b). Thus, methane is an interesting and important target to reduce global warming processes. However, in order to put mitigation strategies in action, knowledge about the sources and sinks of atmospheric methane and the underlying processes leading to methane production and consumption is needed.

Global budget calculations are performed based on different modelling approaches and with increasing accuracy. For this review, two recent calculations are considered (Kirschke *et al.*, 2013; Saunois *et al.*, 2016a). According to these studies, the total global methane emissions are around 560 Tg CH_4/year, while the total sink strength is 550 Tg CH_4/year, resulting in an atmospheric growth of approximately 10 Tg CH_4/year. This growth is with very high confidence linked to anthropogenic activities, which have been estimated to contribute about 60% to global emissions (Ciais *et al.*, 2013; Saunois *et al.*, 2016a).

Focusing on the sources, natural wetlands are the strongest individual source, contributing with 25–32% to global emissions (Fig. 2.1). Moreover, wetlands are assumed to be the main drivers of global inter-annual variability of methane emissions (Ciais *et al.*, 2013). Estimates for freshwaters (lakes, ponds, rivers, estuaries) show still a high uncertainty (Saunois *et al.*, 2016a). Further natural sources are of geological or oceanic origin or from animals (all ≤ 5%). Anthropogenic sources

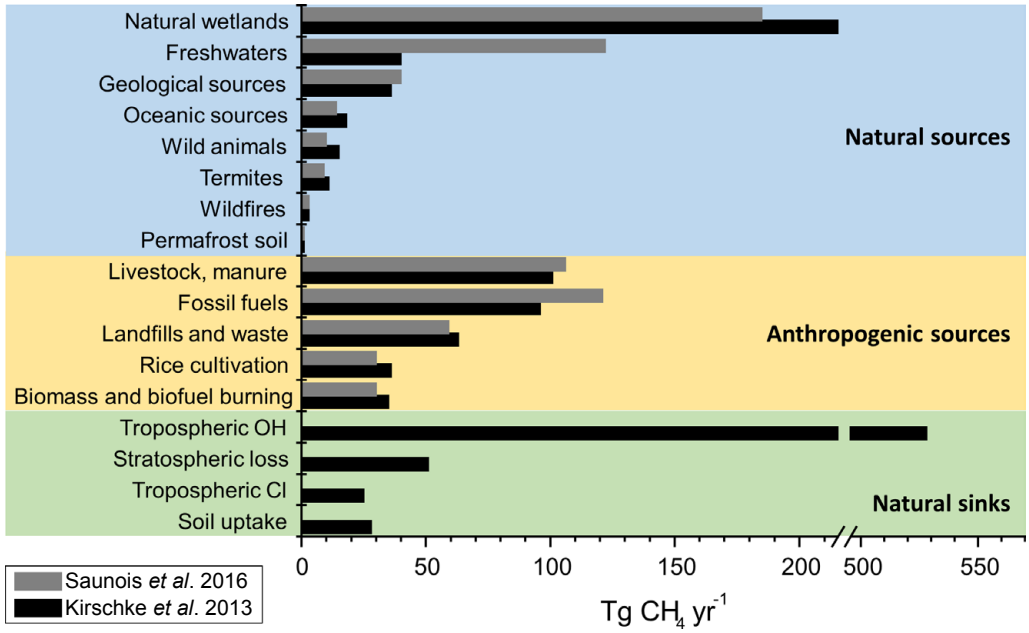

Figure 2.1 Sources and sinks of atmospheric methane. Data were taken from two recent publications, in which emissions were estimated from 2000 to 2009 (Kirschke *et al*., 2013) and 2003 to 2012 (Saunois *et al*., 2016a) based on different modelling approaches. Kirschke *et al*. (2013) presents data as provided in the IPCC report 2013. Different sinks were not resolved by Saunois *et al*. (2016a).

of atmospheric methane contribute between 50% and 60% to the total methane emissions and are predominantly from fossil fuel use and livestock farming (each approximately 15%), followed by landfills and waste treatment (9%), rice cultivation (5%) and biomass and biofuel burning (5%). Most of the atmospheric methane is eliminated by chemical reactions in the atmosphere (Fig. 2.1), whereby the chemical reaction with OH radicals in the troposphere is the predominant process (84%). Moreover, well-aerated soils serve as sink for atmospheric methane, contributing 4% to atmospheric methane oxidation (Kirschke *et al*., 2013). These global budget calculations reveal that soils play an important role, especially as source of atmospheric methane, but also as sink. As sources, natural wetland soils are most relevant, followed by landfill soils and rice paddies. In contrast, well-aerated upland soils represent a relevant sink.

Microbial processes leading to methane production

Besides the classification of methane sources according to their natural or anthropogenic origin, they can be differentiated based on the underlying processes leading to methane formation. Thermogenic, pyrogenic and biogenic sources are differentiated and their source contribution can be estimated based on stable isotope analysis (Ciais *et al*., 2013). Biogenic methane shows the strongest isotopic depletion and is the end product of organic matter degradation in the absence of oxygen or of other oxidants such as nitrate, sulfate or ferric iron. It is produced by methanogenic microorganisms (Conrad, 1996). This process is responsible for methane production in natural wetlands, freshwaters, organic waste deposits (landfills, waste, manure), rice paddies, ruminants, termites and wild animals, so that about 69% of the total atmospheric methane originates from the activity of methanogenic microorganisms (Conrad, 2009). The presence and activity of methanogens and therewith methane emissions are controlled by diverse environmental factors in soils, with substrate availability and concentration of oxygen being among the most relevant factors. These are to some extent linked to other factors such as the concentration and type of organic matter, soil redox potential, availability of electron acceptors, or water

availability and water table. Moreover, temperature, soil pH, availability of nutrients and trace metals, salinity, vegetation, fertilizer and manure additions affect the development of methanogenic communities and their activity and therewith methane emissions (Dalal and Allen, 2008; Dalal *et al.*, 2008; Serrano-Silva *et al.*, 2014).

An update on the diversity of methanogenic Archaea

For decades, methane production was attributed to four specific classes of methanogenic *Euryarchaeota*, the *Methanomicrobia*, *Methanobacteria*, *Methanococci* and *Methanopyri*. The taxonomy, ecology and physiology of the members of these classes has been extensively reviewed (Garcia *et al.*, 2000; Conrad, 2007; Liu and Whitman, 2008; Thauer and Shima, 2008; Thauer *et al.*, 2008; Ferry, 2010; Nazaries *et al.*, 2013; Costa and Leigh, 2014; Serrano-Silva *et al.*, 2014). Many methanogens grow on hydrogen and carbon dioxide as substrates, while members of the genera *Methanosaeta* and *Methanosarcina* grow on acetate. The family *Methanosarcinaceae* is most versatile, i.e. most members are able to grow methylotrophically, for example on methanol or methylated compounds (Oren, 2014). Hydrogenotrophic and aceticlastic methanogenesis are most relevant for biogenic methane production (Conrad, 2005), and the relative contribution of each of these processes to methane production can vary substantially (Conrad, 1999). Methanogenic archaea that are typically found in anoxic soils include *Methanosaetaceae, Methanosarcinaceae* (especially *Methanosarcina*), *Methanomicrobiaceae* (especially *Methanoculleus, Methanomicrobium* and *Methanogenium*), *Methanoregulaceae, Methanospirillaceae, Methanocellaceae, Methanobacteriaceae* (especially *Methanobacterium* and *Methanobrevibacter*) and '*Candidatus* Methanoflorentaceae' (Garcia *et al.*, 2000; Conrad, 2007; Liu and Whitman, 2008). The candidate status of the family '*Ca.* Methanoflorentaceae' indicates that cultured representatives that underwent a formal description are currently lacking.

Recent discoveries indicate that the diversity and physiological versatility of methanogenic microorganisms is actually broader than previously thought, as highlighted by Welte (2018) and reviewed by Kallistova *et al.* (2017). Knowledge has either been gained due to cultivation of novel lineages or based on genome reconstructions from metagenomic data, which were obtained for enrichment cultures of uncultivated groups of methanogens that are merely known from the detection of specific marker genes in environmental samples. These markers are the 16S rRNA gene or the *mcrA* gene, which encodes a subunit of methyl coenzyme M reductase. It is the key enzyme of all methanogenic microorganisms and of some anaerobic methane-oxidizing Archaea. The currently known diversity of methanogenic archaea is illustrated in Fig. 2.2.

Recently discovered methanogenic taxa within the phylum *Euryarchaeota*

Within the *Euryarchaeota* different new groups of methanogens were discovered during the last years. The well-known rice cluster I (RC-I) organisms, which are important methane producers in rice paddies (Lu and Conrad, 2005; Conrad *et al.*, 2006), were brought into culture a decade ago and are in the meantime represented by three different species within the genus *Methanocella*, i.e. *Methanocella paludicola*, *Methanocella arvoryzae* and *Methanocella conradii*, all isolated from rice field soil (Lü and Lu, 2012).

Recently, rice cluster II (RC-II) (Großkopf *et al.*, 1998) has been characterized in more detail based on reconstructed genome data from a population inhabiting thawing permafrost soil. The family name '*Candidatus* Methanoflorentaceae' has been proposed for this group of organisms, belonging to the order *Methanocellales* (Fig. 2.2) (Mondav *et al.*, 2014). Similar as the other *Methanocellales*, '*Candidatus* Methanoflorens stordalenmirensis' is predicted to be hydrogenotrophic. It appears to be an important player in different ecosystems, but especially in cold wetlands (McCalley *et al.*, 2014; Mondav *et al.*, 2014; Kao-Kniffin *et al.*, 2015).

With the description of *Methanomassiliicoccus luminyensis* the first and currently only methanogenic isolate of the class *Thermoplasmata* was identified (Dridi *et al.*, 2012). It represents the new order *Methanomassiliicoccales* within this class (Iino *et al.*, 2013) and was formerly known as rice cluster III (RC-III) (Großkopf *et al.*, 1998). The class *Thermoplasmata* is the first one that harbours a methanogenic order as well as a non-methanogenic order. Besides the isolate *M. luminyensis*, several candidate genera representing *Methanomassiliicoccales*

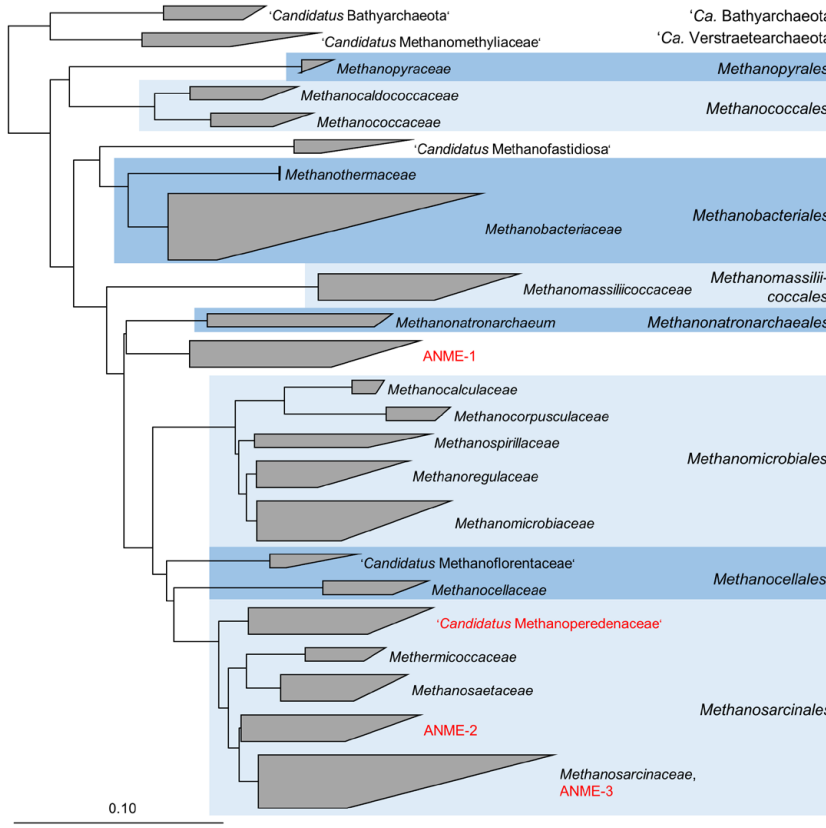

Figure 2.2 16S rRNA gene sequence based phylogenetic tree summarizing the diversity of methanogenic archaea (shown in black) and anaerobic methanotrophic archaea (marked in red). Sequences from type strains and a representative subset of sequences from uncultivated organisms are included in the tree as available in the SSURef_NR99_132_SILVA database. The tree was calculated in ARB using the neighbour joining algorithm with Jukes-Cantor correction and an archaeal filter (1450 nucleotide positions). Sequences were grouped at family level and the different orders of methanogenic *Euryarchaeota* are indicated as far as they have been classified at order level. For predicted methanogens outside of the phylum *Euryarchaeota* the candidate phylum name is given.

have been characterized based on metagenomic sequencing of highly enriched cultures, including '*Candidatus* Methanogranum caenicola', '*Candidatus* Methanomethylophilus alvus', '*Candidatus* Methanoplasma termitum' and '*Candidatus* Methanomassiliicoccus intestinalis' (Borrel *et al.*, 2013a,b; Iino *et al.*, 2013; Lang *et al.*, 2015). These methanogens were mostly obtained from the intestinal tract of humans or animals. 16S rRNA and *mcrA* gene sequence analyses including those from public databases indicate that methanogenic *Methanomassiliicoccales* form two distinct clades, the host-associated and the free-living clade (Paul *et al.*, 2012; Söllinger *et al.*, 2016; Borrel *et al.*, 2017). The free-living clade includes the isolate *Methanomassiliicoccus luminyensis* as well as sequences from diverse terrestrial habitats such as sediments, landfill leachates, wetland soils, hot springs, permafrost sediment, and rice paddies (Borrel *et al.*, 2013b; Iino *et al.*, 2013; Chojnacka *et al.*, 2015; Lang *et al.*, 2015; W. Li *et al.*, 2016; Merkel *et al.*, 2016; Söllinger *et al.*, 2016; Winkel *et al.*, 2018). In contrast to the other methanogenic *Euryarchaeota*, all (meta-)genome sequenced *Methanomassiliicoccales* lack the pathway for CO_2 reduction to methyl coenzyme M and gain energy by a hydrogen-dependent reduction of methanol or methylamines (Lang *et al.*, 2015; Y. Li *et al.*, 2016; Söllinger *et al.*, 2016).

The class *Methanonatronarchaeia* was only recently discovered, including the species *Methanonatronarchaeum thermophilum* and '*Candidatus* Methanohalarchaeum thermophilum' (Sorokin

et al., 2018). They represent the formerly uncultivated halophilic SA1 euryarchaeal group (Eder *et al.*, 2002). As implemented in the names, these organisms are adapted to hypersaline and moderately thermophilic conditions and were obtained from soda lakes. They are not monophyletic to other classes of methanogens, but are most closely related to the class *Halobacteria*. Likewise as the *Methanomassiliicoccales,* these organisms show a methylotrophic lifestyle, but in this case hydrogen or formate serve as electron donors and different C_1-compounds such as methanol or methylamines as acceptor (Sorokin *et al.*, 2017). These organisms lack some genes of the CO_2-reduction pathway, which is relevant for the formation of methane from CO_2.

The uncultivated methanogenic lineage WSA2 (or Arc I), which occurs in a wide range of natural and engineered environments but especially in wastewater treatment plants and marine sediments, is meanwhile represented by one candidate genus, proposed based on metagenome sequence analysis of four WSA2 populations, which were obtained from methanogenic bioreactors treating wastewater (Nobu *et al.*, 2016). It is referred to as '*Candidatus* Methanofastidiosum methylothiophilus' and represents a distinct class, '*Candidatus* Methanofastidiosa' (Nobu *et al* 2016). Likewise, as observed in several of the aforementioned new classes and orders, a complete pathway for CO_2 reduction to methane and for aceticlastic methanogenesis appears to be absent. Instead, these organisms seem to dependent on hydrogen as electron donor and methyl groups obtained from demethylation of methylated thiols, e.g. methylsulfide, as electron acceptors. Moreover, no carbon fixation pathway was identified, and a heterotrophic lifestyle with acetate, malonate or propionate as carbon sources was proposed for these organisms. Furthermore, they lack biosynthetic pathways for several amino acids. These peculiarities have likely contributed to the fact that these methanotrophs eluded cultivation so far, likewise as the host-associated *Methanomassiliicoccales*.

Novel potential methanogenic taxa beyond the phylum *Euryarchaeota*

Besides the isolation of new lineages of methanogens within the *Euryarchaeota*, metagenomic studies point to the existence of methanogens outside the *Euryarchaeaota*. Reconstructed genomes from metagenomic datasets revealed the presence of methanogenetic pathways in representatives from the archaeal candidate phyla '*Candidatus* Bathyarchaeota' and '*Candidatus* Verstraetearchaeota' (Evans *et al.*, 2015; Vanwonterghem *et al.*, 2016). These are representatives of a group of archaea with broad environmental distribution, harbouring species with diverse physiologies and ecological functions, also known as Miscellaneous Crenarchaeotal Group or Group 1.3 archaea (Lloyd, 2015).

Near-complete genome data from two distinct lineages of '*Ca.* Bathyarchaeota' were obtained in samples from formation water of deep coalbed methane wells in Australia (Evans *et al.*, 2015). The genome-sequenced representatives of '*Ca.* Verstraetearchaeota' were assigned to two new candidate genera, i.e. '*Candidatus* Methanomethylicus' and '*Candidatus* Methanosuratus' (Vanwonterghem *et al.*, 2016). They were detected in experimental anaerobic digesters set up with inocula from different natural and engineered anoxic environments with high methane flux (rumen, lake sediment, anaerobic digester and lagoon). From the reconstructed genomes the authors of both studies suggested that the organisms are methylotrophic methanogens. Remarkably for these organisms is the presence of metabolic pathways that appear to enable them to carry out fermentation processes using amino acids, fatty acids or sugars as substrates, a feature that has not been observed among archaeal methanogens. Moreover, Evans *et al.* (2015) stated that it might be possible that these organisms gain energy from anaerobic oxidation of methane. To further validate these predictions and prove the metabolic versatility, it will be necessary to study these organisms in more detail directly in the environment or after enrichment and, if possible, isolation of representative strains. A detailed analysis of the metabolic capabilities appears of particular relevance concerning the methane-cycling capabilities of '*Ca.* Bathyarchaeota', due to the finding that *mcrA* sequences of '*Candidatus* Syntrophoarchaeum', which are similar to those of '*Ca.* Bathyarchaeota', encode an MCR-like protein catalysing the formation of butyl-coenzyme M from butane (Laso-Pérez *et al.*, 2016). Similarly as the organisms sequenced by Evans *et al.* (2015), '*Ca.* Syntrophoarchaeum' possesses an almost-complete methanogenesis-related pathway and four

complete *mcr* gene sets, but grows on butane, which is converted to butyl-coenzyme M, in analogy to the activation of methane to methyl-coenzyme M by anaerobic methanotrophic arachaea (ANME). However, formation of methyl-coenzyme M in the presence of methane was not observed for '*Ca.* Syntrophoarchaeum'. Thus, it can at the moment not be excluded that members of the '*Ca.* Bathyarchaeota' may actually be non-methane alkane oxidizers and the involvement in methane production or possibly oxidation needs to be carefully proven.

The occurrence of these two archaeal groups with methane-cycling potential in nature was further assessed by screening public databases for the presence of 16S rRNA genes in datasets from other studies. Sequences being similar to the sequenced members of both phyla, '*Ca.* Bathyarchaeota' and '*Ca.* Verstraetearchaeota' were indeed detected in different methane-rich habitats including freshwater wetland soils (Vanwonterghem *et al.*, 2016; Narrowe *et al.*, 2017). Vanwonterghem *et al.* (2016) concluded that anoxic conditions, high methane fluxes and a likelihood for increased concentrations of methylated compounds are common characteristics of the habitats in which members of '*Ca.* Verstraetearchaeota' are found. The phylum '*Ca.* Bathyarchaeota' includes diverse non-methanogenic members, which can even be present in methanogenic environments (He, Y. *et al.*, 2016; Lazar *et al.*, 2016; Maus *et al.*, 2018), so the mere detection of 16S rRNA gene sequences representing this phylum is not indicative for the presence of potential methane cycling microorganisms. Conclusions about the presence of these potential methane-cycling microorganisms should thus be drawn carefully and are most reliable if 16S rRNA gene sequences are found that are highly similar to those of the genome sequenced methane-cycling organisms.

Alternatively to the 16S rRNA gene, the *mcrA* gene is a useful target for the detection of methanogenic archaea. The *mcrA* gene was detected in all reconstructed genomes of '*Ca*. Bathyarchaeota' and '*Ca.* Verstraetearchaeota' (Evans *et al.*, 2015; Vanwonterghem *et al.*, 2016), with sequences of '*Ca*. Bathyarchaeota' being clearly distinct from those of the methanogenic *Euryarchaeota,* while those of '*Ca.* Verstraetearchaeota' are quite similar to those of other methanogenic *Euryarchaeota* (Vanwonterghem *et al.*, 2016). The detection of *mcrA* sequences highly similar to those of '*Ca.* Verstraetearchaeota' in metagenomic datasets from terrestrial mud volcanoes and palm oil mill effluent point to a broader distribution of these organisms (Vanwonterghem *et al.*, 2016). Moreover, *mcrA* genes of '*Ca.* Verstraetearchaeota' were found in geothermal spring sediments and at low abundance in a boreal lake sediment (McKay *et al.*, 2017; Rissanen *et al.*, 2017). Even *mcrA* gene expression was proven in these studies. Remarkably, no corresponding 16S rRNA gene sequences of '*Ca.* Verstraetearchaeota' were detected in the respective geothermal spring samples, indicating that the detected *mcrA* sequences are either present in another phylogenetic taxon besides '*Ca.* Vertraeterachaota', or that the 16S rRNA gene based primer was biased concerning the amplification of '*Ca.* Vertraetearchaota'. The *mcrA* genes highly similar to those of '*Ca.* Bathyarchaeota' were found in different high-methane flux environments, including other hydrocarbon seep samples, tar sand tailing ponds, petroleum reservoir sediments, several aquatic environments and geothermal spring sediments (Evans *et al.*, 2015; McKay *et al.*, 2017). Several of these habitats will provide other alkanes such as butane as carbon source, so that the function of this group of archaea in these habitats remains currently unclear.

Methane production in soils under oxic conditions

Recent findings indicate that methane production can also occur under oxic conditions, i.e. in upland soils or wetland soils that become temporarily or partially oxic. However, methanogens have long been considered to be strictly anaerobic and most of them are known to be sensitive to oxygen (Fetzer *et al.*, 1993; Whitman *et al.*, 2014). Despite this assumption, methanogenic archaea have been found in diverse upland soils, including soils from forests, meadows and grasslands, agricultural land, savannas, as well as cold and warm desert and sub-/alpine ecosystems (e.g. Peters and Conrad, 1995; Angel *et al.*, 2012; Aschenbach *et al.*, 2013; Praeg *et al.*, 2014; Hofmann *et al.*, 2016; Hernández *et al.*, 2017; Xie *et al.*, 2017). Moreover, methane production was observed upon incubation of these soils under anoxic conditions, indicating that the activity of the methanogenic archaea can be stimulated under appropriate conditions, and some studies

reported methane production from oxic soils (Teh *et al.*, 2005; Kammann *et al.*, 2009). It is assumed that the activity of methanogens in these soils is temporally and spatially limited, occurring in anoxic microniches, which are formed by the soil structure or are provided by the soil fauna (Conrad, 1995; Kammann *et al.*, 2009, 2017). Their activity may support atmospheric methane-oxidizing bacteria, which rely otherwise on the very low concentrations of atmospheric methane.

Molecular analysis revealed that *Methanocella* and *Methanosarcina* were most commonly detected in upland soils. Besides, *Methanomassiliicoccus*, *Methanobacterium*, *Methanosaeta* and *Methanobrevibacter* were repeatedly detected (e.g. Angel *et al.*, 2011, 2012; Aschenbach *et al.*, 2013; Hu *et al.*, 2013; Praeg *et al.*, 2014; Hofmann *et al.*, 2016; Hernández *et al.*, 2017; Xie *et al.*, 2017). The activity of these genera in upland soils remains largely unexplored. Activity studies of methanogens have only been performed in different drained wetland soils. In oxic paddy soil, the same genera, i.e. *Methanosaeta*, *Methaosarcina*, *Methanobacterium* and *Methanocella,* were shown to be involved in organic matter degradation in a ^{13}C-labelling experiment (Lee *et al.*, 2012). Other studies with paddy soils revealed that oxygen exposure resulted in a strong decrease in *mcrA* gene expression (Yuan *et al.*, 2011; Liu *et al.*, 2018). After the aeration event, several of the above mentioned genera showed the best recovery, i.e. highest *mcrA* gene expression (Yuan *et al.*, 2011; Reim *et al.*, 2017; Liu *et al.*, 2018). In a freshwater wetland from a lakeshore, substantial methane production was reported from the oxygenated soil layer and attributed to the activity of '*Candidatus* Methanothrix paradoxum' (Angle *et al.*, 2017). The genus name *Methanothrix* is supposed to replace the genus name *Methanosaeta* (Garrity *et al.*, 2011), thus the finding is also in agreement with the observation concerning the presence of *Methanosaeta* in upland soils. The activity of this methanogen was assumed to be restricted to anoxic microsites within the overall oxic soil layer. This was concluded from the finding that gene expression analyses of the strain did not indicate the activation of genes involved in oxygen detoxification mechanisms, although this strain possesses a set of such genes.

The presence of genes involved in adaptation mechanisms to oxidative environments was recently analysed systematically in genome sequenced methanogenic archaea and revealed that the methanogens can be divided into two classes, those that have accumulated genes involved in oxygen resistance (*Methanocellales*, *Methanomicrobiales* and *Methanosarcinales*) and those that lack most of these genes (*Methanobacteriales*, *Methanococcales* and *Methanopyrales*) (Lyu and Lu, 2018). In agreement with this classification, nearly all taxa that were found in upland soils or in wetland soils under oxygen stress conditions belong to the group with the higher number of antioxidant features in the genome. Similarly, Lyu and Lu (2018) reported that the taxa with accumulation of antioxidant genes were more frequently detected in microaerophilic or oxic environments, including oceans, rice soils, subsurface soils, and diverse upland and wetland soils. Their observation was based on a meta-analysis, in which the occurrence of publicly available 16S rRNA genes was evaluated. Remarkably, the genera *Methanobrevibacter* and *Methanobacterium*, which were detected in some upland soils, do not belong to the group of methanogens harbouring a diverse set of antioxidant genes. Nevertheless, *Methanobrevibacter* has been shown to remain active in the presence of oxygen, being even able to reduce oxygen, as long as the concentration does not exceed the capacity for its removal (Tholen *et al.*, 2007). This indicates that the methanogenic archaea must have developed different strategies to survive oxic conditions in soils and may contribute to methane production even in environments that are mostly oxic. Methane production under apparently oxic conditions has not only been observed in soils, but also in lakes, where methane production was observed in the oxygenated water column, a phenomenon that is referred to as 'the methane paradox' (Grossart *et al.*, 2011; Bogard *et al.*, 2014; Tang *et al.*, 2014; Donis *et al.*, 2017).

Besides the activity of methanogenic archaea in oxic soils, fungi were recently reported to release methane from methionine as precursor and may contribute to methane production (Lenhart *et al.*, 2012). Moreover, different non-biogenic methane production processes are known to occur in soils (Wang *et al.*, 2013, 2017). These result from degradation processes of organic material, including photodegradation, thermal degradation, oxidation by reactive oxidation species, extracellular oxidative metabolism or inorganic chemical reactions. However, the contribution of these processes to methane

emissions in upland soils or aerated wetland soils remains currently uncertain, but may be rather low (Lenhart *et al.*, 2012; Gu *et al.*, 2016; Wang *et al.*, 2017). The methane-oxidizing bacteria in the soils may be able to metabolize most of this methane before it reaches the atmosphere. Further work is needed to assess the importance of these different processes in the diverse continental ecosystems.

Microbial methane oxidation by aerobic methanotrophic bacteria

Biological aerobic methane oxidation is exclusively performed by bacteria. The methanotrophic bacteria, which gain carbon and energy from the oxidation of methane, inhabit diverse terrestrial, aquatic and marine habitats. Terrestrial ecosystems that are known to act as sources for atmospheric methane host diverse methane-oxidizing bacteria. These methanotrophic bacteria are found in high Arctic and tundra wetlands, peat bogs, rice paddies, landfill covers, sewage sludge and floodplains (Knief, 2015). In these ecosystems, aerobic methanotrophic bacteria usually inhabit the oxic/anoxic interfaces, where they oxidize the methane that is released by the methanogenic archaea. The filter capacity of the methanotrophic bacteria can lead to more than 80% reduction in methane emissions, especially in oceans, freshwaters and rice paddies, while the filter effect appears less efficient in wetlands and landfill soils (Conrad, 1996; Reeburgh, 2003).

In upland soils aerobic methanotrophic bacteria are responsible for atmospheric methane oxidation (Bender and Conrad, 1992; Dunfield, 2007; Kolb, 2009; Knief, 2015). They live on the expense of this atmospheric methane and, if available, endogenously produced methane in soil (see previous section). Moreover, some of them may profit from multi-carbon compounds (Pratscher *et al.*, 2011, 2018). The presence and activity of methanotrophic bacteria in soils is controlled by diverse environmental factors. As for the methanogenic archaea, substrate availability and concentration of oxygen are considered to be the most important factors, while water availability and water table, soil pH, availability of nutrients and trace metals (especially copper), temperature, salinity, vegetation, fertilizer and manure additions will further affect the abundance and activity of these microorganisms (Dalal and Allen, 2008; Dalal *et al.*, 2008; Semrau *et al.*, 2010; Aronson *et al.*, 2013; Serrano-Silva *et al.*, 2014). While the effect of these environmental factors on methane oxidation rates has been studied quite intensively, knowledge about the responses of the microbial groups involved in methane oxidation is less advanced.

An update on the diversity of cultivated aerobic methanotrophic bacteria

Aerobic methanotrophic bacteria are found within three bacterial classes, the *Alphaproteobacteria*, *Gammaproteobacteria* and *Methylacidiphilae*, the latter being members of the phylum *Verrucomicrobia*. An overview of the currently known diversity of cultivated methanotrophic bacteria is given in the phylogenetic tree (Fig. 2.3). Besides the classification of aerobic methanotrophic bacteria based on their phylogeny, grouping into type I (*Gammaproteobacteria*), type II (*Alphaproteobacteria*) and sometimes type III (*Methylacidiphilae*) methanotrophs is quite common, especially in cultivation-independent studies. This grouping into different types is not meant to encode specific phylogenetic information. The type I methanotrophs are further differentiated into type Ia to Id methanotrophs, whereby type Ia and Ib represent *Methylococcaceae*, type Ic *Methylothermaceae* and type Id an uncultivated lineage of methanotrophs, defined based on their *pmoA* sequences (Knief, 2015). Major characteristics of these methanotrophs were compiled in recent reviews (Knief, 2015; Dedysh and Knief, 2018). Thus, the focus in this section will be on recently obtained methanotrophic isolates that represent new genera of methanotrophic bacteria and their occurrence in soils.

Within the group of methanotrophic *Gammaproteobacteria*, a couple of different new isolates or enrichment cultures were recently obtained. *Methyloterricola oryzae* strain 73a^T was isolated from the lower part of stems from rice plants and is the first cultured representative of rice paddy cluster 1 (RPC1) (Frindte *et al.*, 2017). The isolate is a typical member of the *Methylococcaceae*, most closely related to the genera *Methylococcus* and '*Candidatus* Methylospira' (Fig. 2.2). RPC1 represents methanotrophic bacteria that are frequently detected

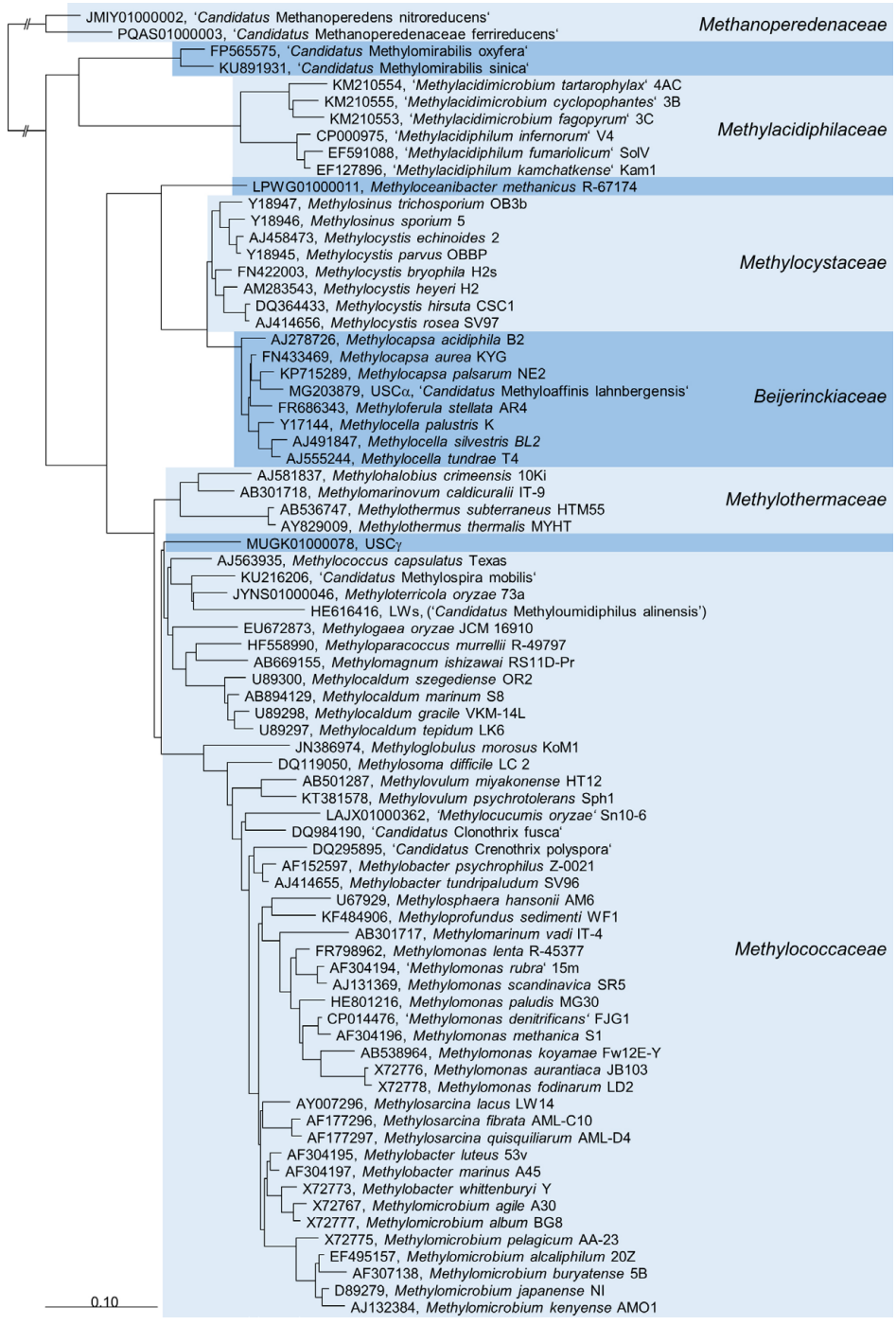

Figure 2.3 16S rRNA gene sequence based phylogenetic tree summarizing the diversity of aerobic methanotrophic bacteria and anaerobic methanotrophic bacteria and archaea. All type strains of validated aerobic methanotrophic species are included as well as methanotrophs that have been described as new species but have not yet been formally validated (names are hyphenated). Moreover, methanotrophs with candidate status are included, which are mostly available as enrichment culture, but not as pure culture isolates. This rules out their validation as new species based on current regulations. The tree was calculated in ARB using the neighbour joining algorithm with Jukes-Cantor correction and a bacterial filter (1565 nucleotide positions). Shorter sequences were added using the ARB parsimony quick-add tool. The assignment of the strains to different families is indicated as far as a classification at this taxonomic rank has been done.

in rice paddies, but also in aquatic ecosystems and wetlands (Knief, 2015). The cluster has been defined based on the detection of *pmoA* sequences. The *pmoA* gene encodes a subunit of the particulate methane monooxygenase and is the most widely used molecular marker for aerobic methanotrophic bacteria due to its presence in the fast majority of aerobic methanotrophs (Knief, 2015).

The methanotrophic isolate strain Sn10-6 is proposed to represent a new genus and species, '*Methylocucumis oryzae*' (Rahalkar *et al.*, 2016; Pandit *et al.*, 2018). It is distantly related to other type Ia methanotrophic bacteria and it was isolated from the rice rhizosphere. The preferred habitat of this genus remains currently largely unknown, because highly similar 16S rRNA or *pmoA* gene sequences are not present in the NCBI nucleotide collection database. A more specific search for similar sequences (>95% sequence identity) of these two marker genes in datasets obtained from rice ecosystems via high-throughput amplicon sequencing resulted in a few hits for both genes in two studies (Lee *et al.*, 2015; J. Liu *et al.*, 2017). In several other studies analysing paddy soil or the rice rhizosphere, it was not detected despite the higher sensitivity that is achieved when using next generation sequencing technologies (e.g. Lüke and Frenzel, 2011; Knief *et al.*, 2012; Ahn *et al.*, 2014; Vaksmaa *et al.*, 2017c; Shiau *et al.*, 2018). Such a rare detection of a newly isolated genus in cultivation-independent studies has previously been seen for a few other genera, mostly from marine environments (Knief, 2015). The relevance of these genera for global methane cycling remains thus largely unclear.

Another new candidate genus representing methanotrophic *Gammaproteobacteria*, '*Candidatus* Methylospira', could not yet be obtained in pure culture and has therefore the '*Candidatus*' status (Danilova *et al.*, 2016b). It was enriched from a *Sphagnum* dominated peat bog and is a representative of the *pmoA* OSC cluster, which is closely related to RPC I, as reflected by the relatedness of the 16S rRNA gene sequences of '*Candidatus* Methylospira mobilis' and *Methyloterricola oryzae* (Fig. 2.3). Sequences of this cluster were detected in different fen and bog ecosystems, but also in an organic-rich and a mineral soil. The study of Danilova *et al.* (2016b) reported its presence in different freshwater and lake sediments, thus it appears to colonize predominantly different aquatic and wetland ecosystems. Moreover, it was found as a dominant member within the methanotrophic community in a lichen-dominated patch of a boreal peatland ecosystem (Danilova *et al.*, 2016a). Characteristic for this genus are the spiral-shaped cells, which are so far unique among methanotrophs, and the preference to growth under micro-oxic conditions. The detection of this genus in different wetlands suggests that a microaerophilic lifestyle along with motility may be an important trait for this genus to establish a population in peatlands.

Recent insights obtained for major uncultivated groups of methanotrophic bacteria

Besides '*Candidatus* Methylospira mobilis', which exists as enrichment culture and has been described in detail, some further taxa of putative methanotrophs with *Candidatus* status have been proposed to exist. They were characterized based on information from genome reconstructions derived from metagenomic data. Considering the necessary requirements to refer to a taxon as *Candidatus*, these organisms should not be termed *Candidatus*, likewise as several of the above mentioned methanogens, because limited information is available concerning structural, metabolic or reproductive features. In some cases, a nearly complete 16S rRNA sequence is also lacking, although the availability of this sequence is currently another prerequisite for taxa with *Candidatus* status according to taxonomic rules (Murray and Stackebrandt, 1995). All groups of uncultivated methanotrophs that include a recently described strain or an uncultivated but genome sequenced representative are compiled in Table 2.1.

Upland soil cluster α (USCα) and MHP clade

The best-described group of uncultivated aerobic methanotrophic organisms is USCα, which is known as major player involved in atmospheric methane oxidation in upland soils (Dunfield, 2007; Kolb, 2009; Knief, 2015). For a genome analysis of this group of methanotrophs, cells were obtained upon artificial enrichment from forest soil samples known to harbour these organisms as dominant group of methanotrophs (Pratscher *et al.*, 2018). Phylogenetic placement of the 16S rRNA

Table 2.1 Recent insights obtained for major *pmoA* and *pmoA*-like sequence clusters

pmoA/*amoA* sequence cluster	Gained knowledge	Predominant habitat	Reference
RPC1	Isolate *Methyloterricola oryzae* strain 73a^T characterized, member of the *Methylococcaceae*	Rice paddies and aquatic habitats	Frindte *et al*. (2017)
OSC	Enrichment culture '*Candidatus* Methylospira mobilis' characterized, member of the *Methylococcaceae*	Peat bogs	Danilova *et al*. (2016b)
USCα	Genome reconstruction of a representative organism, member of the *Beijerinckiaceae*, proposition of the name '*Candidatus* Methyloaffinis lahnbergensis'	Upland soils, caves and lava tubes	Pratscher *et al*. (2018)
USCα, MHP clade	Genome reconstruction of two representative organisms, member of the *Beijerinckiaceae*	Peatlands	Singleton *et al.* (2018)
USCγ	Genome reconstruction of a representative organism, member of the *Gammaproteobacteria*	Upland soils, caves and lava tubes	Edwards *et al*. (2017)
LWs	Genome reconstruction of a representative organism, member of the *Methylococcaceae*, proposition of the name '*Candidatus* Methyloumidiphilus alinensis'	Aquatic habitats	Rissanen *et al*. (2018)
Crenothrix (*pmoA*/*amoA*)	Sequence cluster with 'unusual *pmoA*' of Crenothrix represents *amoA* sequences of *Nitrospira* species capable of comammox; Crenothrix harbour gammaproteobacterial *pmoA* gene sequences	Diverse terrestrial and aquatic habitats	Van Kessel *et al*. (2015), Daims *et al*. (2015), Oswald *et al*. (2017)

gene sequence and multi-locus sequence analyses indicated that this methanotroph represents a new genus within the family *Beijerinckiacea*, most closely related to *Methylocapsa* species, as already assumed based on *pmoA* gene sequence analysis (Fig. 2.4) and *pmo* operon analyses (Ricke *et al*., 2005). Furthermore, genome analysis revealed the presence of a complete set of genes needed for C_1 metabolism and confirmed the possibility to grow on acetate as carbon source, as already proposed in an earlier study based on stable isotope incorporation with acetate as carbon substrate (Pratscher *et al*., 2011). With the availability of a first known 16S rRNA gene sequence of USCα, the global distribution of this organism was reassessed, largely confirming *pmoA*-based findings, i.e. the recovery from diverse upland soils, in particular forest soils. Interestingly, highly similar 16S rRNA sequences were also recovered from subterranean environments, including caves and lava tubes, where they contributed up to 10% to the bacterial community composition, while this is ≤ 1% in upland soils (Kolb *et al*., 2003; Pratscher *et al*., 2018). The authors propose the name '*Candidatus* Methyloaffinis lahnbergensis' for this group of organisms.

Two further reconstructed genomes for USCα organisms were obtained from metagenomic data from permafrost soils (Singleton *et al*., 2018). Here, the USCα methanotrophs appeared to be the predominant methanotrophs in palsa and were also abundant in the thawed bog samples. The palsa was mainly oxic and methane production was reported to be minimal (McCalley *et al*., 2014), so that atmospheric methane oxidation by USCα is conceivable, while the detection of USCα in a bog sample is rather uncommon for USCα sequence types, with the exception of the MHP clade. The MHP clade is a subgroup within the large USCα cluster, which is typical for peatlands (Knief, 2015). Indeed, the most closely related *pmoA* sequences of the USCα contigs given in the study of Singleton *et al.* (2018) indicate that their USCα genomes represent this MHP clade. Thus, they represent a distinct subgroup of USCα methanotrophs compared to the organisms analysed by Pratscher *et al.* (2018). Analysis of the two reconstructed genomes

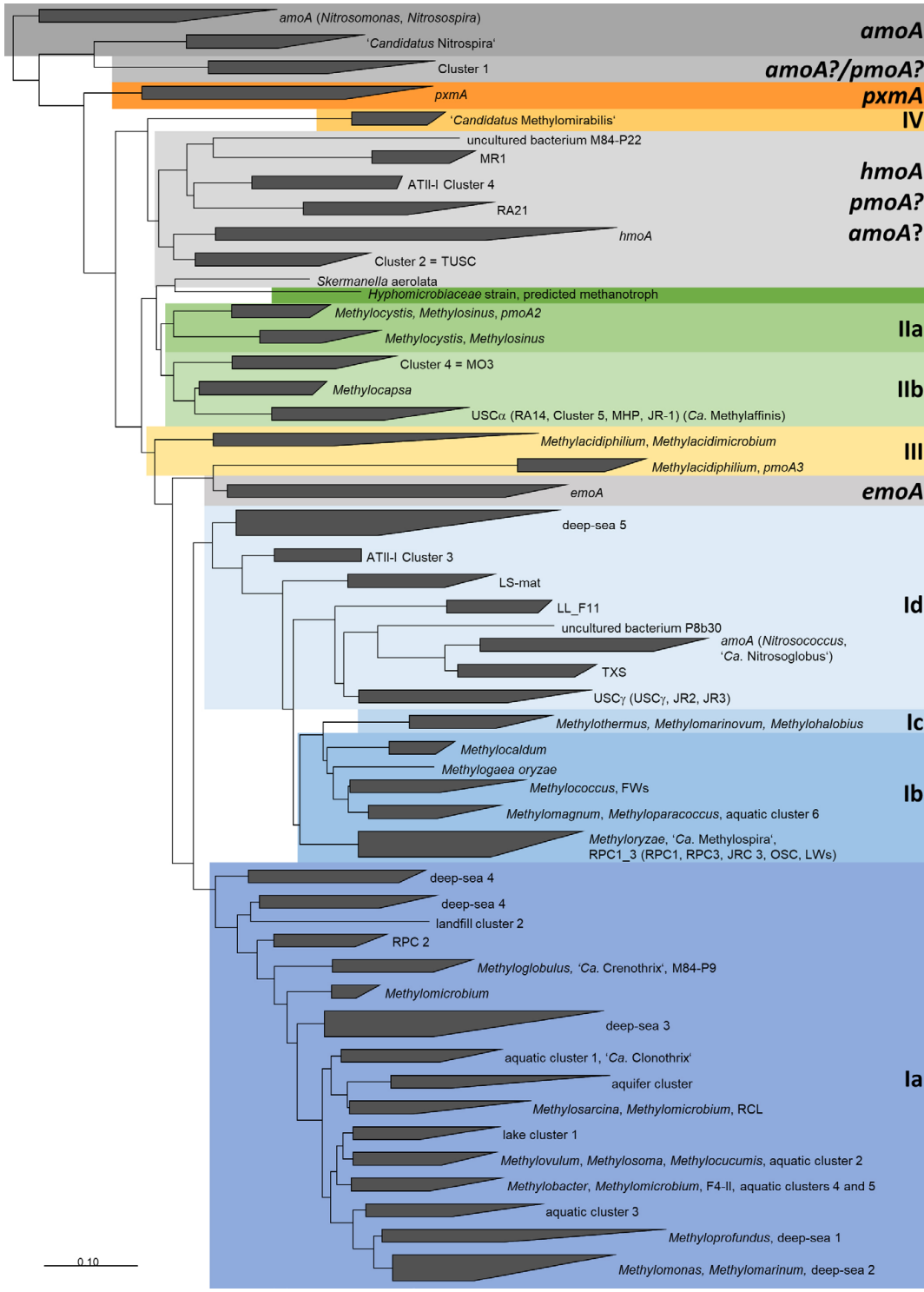

Figure 2.4 Phylogenetic tree showing the diversity of cultured methanotrophs and defined clusters of uncultivated aerobic methanotrophic bacteria based on *pmoA* gene sequence analysis. A backbone tree was used as presented earlier (Knief and Dedysh, 2018) and updated with sequences of recently published novel groups of methanotrophs using the ARB parsimony quick-add tool. The different groups of methanotrophic bacteria are labelled according to their grouping into type I to type IV.

confirmed the relationship of these USCα methanotrophs to *Methylocapsa* species. Furthermore, the presence of genes for pXMO homologue and a lanthanide-dependent XoxF-type methanol dehydrogenase as well as the absence of genes for a calcium-dependent MxaFI-type methanol dehydrogenase, which were reported by Pratscher *et al.* (2018), were confirmed in this study based on both genomes. Additionally, Singleton *et al.* (2018) report the presence of hydrogenase genes and genes involved in carbon monoxide oxidation, suggesting further metabolic versatility of these organisms besides a potential for growth on acetate. A hydrogenotrophic lifestyle was just recently reported for methanotrophic *Verrucomicrobia* (Carere *et al.*, 2017; Mohammadi *et al.*, 2017). The presence and activity of hydrogenases in methanotrophs is known since a long time (e.g. Chen and Yoch, 1987; Hanczár *et al.*, 2002). They were assumed to contribute to the generation of reductants, e.g. for methane monooxygenase, but not considered to allow growth under chemolithotrophic conditions, which demands a pathway for CO_2 fixation. Thus, it may only be an option for those type Ib methanotrophs that possess the capability to assimilate carbon via the Calvin cycle. While Pratscher *et al.* (2018) could not reconstruct a complete Calvin cycle for their organism, Singleton *et al.* (2018) report the presence of all relevant genes. As in other examples, the relevance of this predicted metabolic capability remains to be proven. Similarly, the involvement of the MHP clade in atmospheric methane oxidation has not yet been demonstrated. The study of Singleton *et al.*, 2018 included metatranscriptomic analyses, which did not reveal strong *pmoA* gene expression for these methanotrophs, so that the conditions under which they may be actively involved in methane oxidation in peatlands remain currently unclear.

Upland soil cluster γ

Likewise as for USCα, a reconstructed genome sequence was recently reported for USCγ (Edwards *et al.*, 2017). This *pmoA* sequence cluster is also known to be involved in atmospheric methane oxidation, but more frequently detected in pH neutral upland soils, while USCα is predominant in acidic soils (Knief *et al.*, 2003; Knief, 2015). A draft genome of a representative of this group of organisms was reconstructed from metagenomic data obtained from a mineral cryosoil sample. Only a partial 16S rRNA gene sequence is available, which indicates that USCγ methanotrophs are members of the class *Gammaproteobacteria* (Fig. 2.3). In the SILVA database used for tree reconstruction, the sequence clusters most closely to sequences of uncultured organisms, which form a branch separate from known families of *Gammaproteobacteria*. Interestingly, these closely related 16S rRNA sequences from environmental samples indicate its presence in caves and lava tubes, likewise as reported for USCα. Besides, some sequences were recovered from soils, especially from cold ecosystems. This is in agreement with the detection of the USCγ *pmoA* sequences, which were predominantly found in pH neutral and alkaline soils, and in soils from cold or dry ecosystems (Knief, 2015). Carbon assimilation of this group of organisms remains currently enigmatic, as neither a complete ribulose monophosphate pathway nor a complete serine cycle was reconstructed from the available genomic data. The presence of Calvin cycle genes, which could be another alternative for carbon assimilation, was not evaluated by Edwards *et al.* (2017).

Lake Washington cluster (LWs)

Another methanotrophic organism, proposed as '*Candidatus* Methyloumidiphilus alinensis', has recently been described based on genome reconstructions from a metagenomic dataset, which was obtained from a small oxygen-stratified humic lake (Rissanen *et al.*, 2018). A 16S rRNA gene sequence is not available from the reconstructed genome. Its phylogenetic placement was assessed based on a genomic comparison including a number of different genes, which indicated its relatedness to the genome sequenced *Methyloterricola oryzae* strain 73a^T. However, as other related organisms such as '*Candidatus* Methylospira mobilis' are not yet genome sequenced, its exact phylogenetic placement remains vague. Interestingly, its *pmoA* sequence indicates that the organism is a representative of the LWs cluster, which contains sequences from several different studies and is known to represent predominantly methanotrophs that inhabit freshwater lakes (Dumont *et al.*, 2014; Knief, 2015).

The Crenothrix cluster and a re-evaluation of the phylogenetic placement of Crenothrix *pmoA* sequences

A sequence cluster distantly related to *pmoA* sequences as well as to *amoA* sequences of nitrifying bacteria has been identified as Crenothrix cluster, based on the finding that *pmoA* sequences of the methane-oxidizing '*Candidatus* Crenothrix polyspora' fall into this cluster (Stoecker *et al.*, 2006). However, the identity and metabolic potential of the organisms represented by this sequence cluster has to be questioned based on two recent findings. First, different *Nitrospira* isolates were identified that possess an *amoA* gene with a sequence falling into this Crenothrix cluster (Daims *et al.*, 2015; van Kessel *et al.*, 2015). These *Nitrospira* species oxidize ammonia to nitrate, a process referred to as 'complete ammonium oxidation to nitrate', or comammox. This finding suggests that the sequence cluster is representing comammox bacteria of the genus *Nitrospira*. Second, a recent study reanalysed samples from the waterworks sand filter system, in which the 'unusual *pmoA*' sequences assigned to '*Ca.* Crenothrix polyspora' had initially been found. Genome reconstructions from metagenomic data revealed that the system harbours *Nitrospira* species with the comammox type of *amoA* sequence, and *Crenothrix* species with a gammaproteobacterial *pmoA* sequence (Oswald *et al.*, 2017). Such *pmoA* sequences were also detected in the initial study by Stoecker *et al.* (2006), but not linked to '*Ca.* Crenothrix polyspora' due to their low abundance. Taken together, these findings indicate that the 'Crenothrix cluster' represents *amoA*-like sequences of comammox organisms, while Crenothrix species harbour *pmoA* sequences similar to those of methanotrophic *Gammaproteobacteria*. They are actually similar to the *pmoA* sequences of *Methyloglobulus morosus* (Fig 2.4).

Besides the detection of a Crenothrix methanotroph with a *pmoA* sequence similar to that of *M. morosus*, Oswald *et al.* (2017) reported the existence of another '*Ca.* Crenothrix polyspora' organism from a stratified lake, which has a gammaproteobacterial *pmoA* sequence that is only distantly related to sequences of cultivated type Ia methanotrophs. Instead, its *pmoA* sequence clusters most closely to *pmoA* sequences of diverse uncultivated type Ia methanotrophs, several of them obtained from freshwater methane seeps. However, it does not consistently fall into a specific well-known cluster of uncultivated methanotrophs. Therefore, it is not highlighted in the phylogenetic tree in Fig. 2.4, where it is part of lake cluster 1. This inconsistent clustering is explained by a high number of sequences with equal similarities to each other among the type Ia methanotrophs. Thus, the clustering of sequences varies to some extent in dependence on the dataset and algorithm used for tree calculation. This also explains why the combination of sequences into larger clusters as presented in Fig. 2.4 is not in full agreement with trees shown in previous studies (Dedysh and Knief, 2018; Knief, 2015). Oswald *et al.* (2017) speculate that the freshwater lake Crenothrix has acquired the *pmoCAB* operon laterally from another methanotrophic *Gammaproteobacterium*, as the operon is flanked by transposase genes.

Evidence for methanotrophs in genera not yet known to include aerobic methanotrophic bacteria

The diversity of methanotrophic *Alphaproteobacteria* has recently been extended with the discovery of the methanotrophic strain '*Methyloceanibacter methanicus*' strain R-67174, a member of the order *Rhizobiales* (Vekeman *et al.*, 2016). Besides the *Methylocystaceae* and *Beijerinckiacae*, it represents a third group of methanotrophs within this order (the genus has not yet been classified at class level). The strain was isolated from a marine sediment sample. While the genus *Methyloceanibacter* is known to be methylotrophic, '*Methyloceanibacter methanicus*' strain R-67174 is currently the only known methanotrophic species and strain within this genus. This is the first example of a methanotroph within a non-methanotrophic though methylotrophic genus. The methane oxidation capacity of this strain is realized by the presence of a soluble methane monooxygenase, while genes encoding a membrane bound methane monooxygenase, which occurs almost consistently among methanotrophic bacteria, are absent. Besides *Methylocella* and *Methyloferula*, it is thus the third genus that possesses only the soluble form of the enzyme methane monooxygenase. The *mmoX* gene, which serves as molecular marker for methanotrophs harbouring a soluble methane monooxygenase, was related to those of *Methylocella* and *Methyloferula*, which are both representatives

of the methanotrophic *Beijerinckiacea*. The authors speculate that *Methyloceanibacter* may have acquired the genes encoding the soluble methane monooxygenase by horizontal gene transfer. The occurrence of the genus *Methyloceanibacter* appears to be largely limited to marine environments, all isolates were obtained from marine environments (Takeuchi *et al.*, 2014; Vekeman *et al.*, 2016), and closely related 16S rRNA gene sequences were predominantly detected in marine environments. This is in agreement with the general finding that most marine methanotrophs are clearly distinct from methanotrophic taxa found in terrestrial or aquatic environments (Knief, 2015; Vekeman *et al.*, 2016). Thus, this methanotroph may not play a major role in soil. However, the observation that individual methylotrophs gain the capability to oxidize methane by acquiring genes encoding the soluble or particulate methane monooxygenase may apply to terrestrial microorganisms as well, especially if microorganisms reside in habitats where methanol as well as methane are available as carbon and energy sources, thus supporting the growth of methylotrophic and methanotrophic microorganisms.

Evidence for putative methanotrophic strains in another non-methanotrophic genus comes from the metagenomic study of Singleton *et al.* (2018), in which reconstructed genomes related to the photoheterotrophic *Rhodomicrobium* spp., members of the *Hyphomicrobiaceae*, were reported to harbour operons for both, the particulate and soluble methane monooxygenase. The *pmoA* sequences found in this group of organisms cluster basal to those of the *Methylocystaceae* and *Beijerinckiaceae* (Fig. 2.4) and reflect thus the 16S rRNA gene based phylogeny. Similarly, the MmoX sequences formed a novel cluster related to the *Beijerinckiaceae*. Likewise as some other alphaproteobacterial methylotrophs, this *Hyphomicrobiaceae* strain has genes for a thiol-dependent pathway for formaldehyde oxidation and the necessary equipment to perform carbon assimilation via the Calvin cycle. Furthermore, hydrogenase genes and a complete dissimilatory sulphate reduction pathway were identified, with *dsr* genes similar to those of *Rhodomicrobium*, indicating a broader metabolic versatility also for this group of organisms. In public sequence databases, its *pmoA* sequence type was available from a couple of metagenomes, indicating the presence in wetlands including peat and bog ecosystems. Moreover, 16S rRNA gene sequences related to *Rhodomicrobium* or *Hyphomicrobium* were detected in different ^{13}C-methane stable isotope labelling studies performed in peatlands (Morris *et al.*, 2002; Gupta *et al.*, 2012; Putkinen *et al.*, 2014; Deng *et al.*, 2016). Owing to the methylotrophic lifestyle realized by some members of the *Hyphomicrobiaceae*, labelling of these organisms was assumed to be the result of cross-feeding on partially oxidized C_1 compounds in such labelling experiments.

The results obtained for the *Mehyloceanibacter* isolate and the genome information of the *Hyphomicrobiaceae* strain suggest that methane oxidation must be more widespread among the *Alphaproteobacteria* than previously thought. As for USCα (family *Beijerinckiaceae*), these organisms appear to be metabolically more versatile than the *Methylocystaceae*, so that their impact on methane cycling in an ecosystem cannot yet be assessed, but it deserves more detailed studies to evaluate under which conditions these organisms may contribute to the methane oxidation process. Singleton *et al.* (2018) reported very weak expression of the genes for methane monooxygenases by the *Hyphomicrobiaceae* strain, based on metatranscriptomic data.

Oxygen dependence of aerobic methanotrophic bacteria

Methanotrophic bacteria are considered to be obligately aerobic due to their need of oxygen for respiration and for methane oxidation by the methane monooxygenase. However, studies accumulate that report the presence of aerobic methanotrophic *Gammaproteobacteria* in habitats with very low oxygen concentrations, especially in suboxic and anoxic layers or the sediments of stratified lakes (e.g. Biderre-Petit *et al.*, 2011; Blees *et al.*, 2014; Kojima *et al.*, 2014; Crevecoeur *et al.*, 2015; Hernandez *et al.*, 2015; Oswald *et al.*, 2016; Martinez-Cruz *et al.*, 2017; Naqvi *et al.*, 2018; Singleton *et al.*, 2018). In particular type Ia methanotrophs are consistently detected in these studies. More specifically, these are often reported to represent *Methylobacter* species. A specific assignment to the genus *Methylobacter* may in some cases be questionable, because the different species of *Methylobacter* are not forming monophyletic clusters in 16S rRNA and *pmoA* based trees, so that diverse sequence types exist in public databases that are termed 'uncultured *Methylobacter*'.

Such *pmoA* sequences cluster with the recently detected genera *Methylovulum* and *Methylosoma*, but are also similar to those of *Methylomicrobium* and *Methylosarcina*, or they fall into major groups of uncultivated organisms, representing the *pmoA* lake cluster or different aquatic clusters (Dumont *et al.*, 2014; Knief, 2015). Thus, the adaptation to low-oxygen conditions is most likely not strictly limited to the genus *Methylobacter*.

Focusing on soils, *Methylobacter* was reported to be the dominant active methanotroph with increasing depth and thus decreasing oxygen concentrations in an arctic peat soil (Tveit *et al.*, 2014). Moreover, *Methylobacter* was the dominant active methanotroph in the anoxic zone just below the oxic/anoxic interface in a rice paddy soil microcosm. Actually, a higher *pmoA* transcript to gene ratio was observed in the anoxic soil layer compared with the oxic top soil layer, and highest *pmoA* transcription was observed at the interface (Reim *et al.*, 2012). These findings indicate that *pmoA* gene expression and, linked to it, the activity of aerobic methanotrophic bacteria under microaerophilic or even anoxic conditions is not restricted to aquatic ecosystems, but occurs also in different wetland soils. Thus, aerobic methanotrophs in different ecosystems appear to be less dependent on (high) oxygen concentrations and oxygen availability may be a less strict regulatory factor for the occurrence and activity of at least some aerobic methanotrophs than initially thought.

In agreement with these observations is the enrichment and isolation of methanotrophs from lake sediment and a peat bog ecosystem that grow preferentially under microaerophilic conditions, i.e. *Methylosoma difficile, Methyloglobulus morosus* and '*Candidatus* Methylospira mobilis' (Rahalkar *et al.*, 2007; Deutzmann *et al.*, 2014; Danilova *et al.*, 2016b). While the adaptation mechanisms to microaerophilic conditions of these specific taxa are not yet known, different mechanisms are known from other aerobic methanotrophic bacteria. One strategy appears to be the use of alternative terminal oxidases with high affinity to oxygen. The presence of a cytochrome *bd* oxidase (K_m value for oxygen between 3 and 8 nM) has been reported for a strain of the family *Methylothermaceae*, related to *Methylohalobius crimeensis* and *Crenothrix* (Skennerton *et al.*, 2015; Oswald *et al.*, 2017). The genes for this oxidase were found in reconstructed genome information, assembled from metagenomic datasets. The activity and affinity of this oxidase in aerobic methanotrophic bacteria remains to be studied to validate its involvement in adaptation of methanotrophs to oxygen-limited conditions.

Furthermore, several aerobic methanotrophs have the genetic equipment to perform anaerobic respiration by denitrification. Methane-dependent denitrification activity using nitrate or nitrite as substrate was reported for '*Methylomonas denitrificans*' and *Methylomicrobium album* BG8, respectively (Kits *et al.*, 2015a,b) and relevant genes have been found in further gammaproteobacterial methanotrophs including *Methylobacter* or *Crenothrix* (Campbell *et al.*, 2011; Kalyuzhnaya *et al.*, 2015; Skennerton *et al.*, 2015; Oswald *et al.*, 2017). Moreover, the alphaproteobacterial *Methylocystis* sp. strain SC2 was shown to perform complete denitrification from nitrate to dinitrogen under anoxic conditions in the presence of methanol as growth substrate (Dam *et al.*, 2013). Since some *Methylocystis* strains are known to be facultative methanotrophs, even though with reduced growth capacities compared with growth on methane (Belova *et al.*, 2011; Im *et al.*, 2011), the combination of facultative methanotrophy with denitrification could be a way to overcome oxygen limitation for members of this genus. Besides anaerobic respiration, fermentation has recently been reported as an adaptation strategy for *Methylomicrobium alcaliphilum* strain 20Z (Kalyuzhnaya *et al.*, 2013). The authors observed the production of different organic acids in the presence of very low oxygen concentrations, which were still sufficient to perform methane oxidation. Finally, aerobic methanotrophic bacteria were reported to survive extended periods of anoxic conditions (Roslev and King, 1994).

All these observations demonstrate that aerobic methanotrophic bacteria have developed different strategies to live or at least to survive under conditions of severe oxygen limitation. However, the relevance of these different mechanisms under *in situ* conditions remains currently largely unclear. Likewise, mechanisms to overcome the need for oxygen for the initial methane oxidation step remain unknown (Chistoserdova, 2015). A putative oxygen scavenging protein was recently discussed in this context, due to the fact that gene expression of a cyanoglobin homologue was upregulated

under hypoxic conditions and detected in '*Methylomonas denitrificans*' and the reconstructed genome of the *Methylothermaceae* strain (Kits *et al.*, 2015b; Skennerton *et al.*, 2015). Since bacterial globins can have different functions (Vinogradov *et al.*, 2013), this protein and its possible role in methanotrophs remains to be studied.

For freshwater ecosystems, further possibilities are under discussion to explain the activity of aerobic methanotrophs under anoxic conditions: (i) methanotrophic bacteria living in association with phototrophic microorganisms that produce oxygen, which is instantaneously consumed and thus not detectable (Milucka *et al.*, 2015; Oswald *et al.*, 2015), linked to it (ii) oxygen concentrations are below the detection limit of standard oxygen sensors and thus not detectable (Blees *et al.*, 2014), (iii) oxygen may episodically be transported into anoxic layers and therewith support aerobic methanotrophs (Blees *et al.*, 2014), (iv) the sedimentation of inactive cells from oxic layers (Schubert *et al.*, 2006), and (v) perhaps the use of alternative electron acceptors such as Mn(IV) or Fe(III) (Oswald *et al.*, 2016). However, these hypotheses remain to be proven and different explanations may be valid under different conditions.

Anaerobic methanotrophic archaea and bacteria

Besides the aerobic methanotrophic bacteria, anaerobic methane-oxidizing archaea and bacteria contribute to the reduction of methane emissions in ecosystems that act are sources for atmospheric methane. This is well known to be of particular relevance in marine ecosystems, where >90% of the produced methane is oxidized by anaerobic methanotrophs (Knittel and Boetius, 2009). Knowledge about the filter effect of anaerobic methanotrophs in terrestrial ecosystems is still limited, although evidence is accumulating concerning the relevance of this process. The process of anaerobic methane oxidation has been detected in diverse soils, especially in different natural wetlands (e.g. Smemo and Yavitt, 2007; Gupta *et al.*, 2012; Hu *et al.*, 2014; Gauthier *et al.*, 2015; Segarra *et al.*, 2015).

First global estimates about the relevance of anaerobic methane oxidation are available for natural wetlands, but still imprecise. According to two independent studies, between 4 and 200 Tg of methane is oxidized by these organisms per year (Hu *et al.*, 2014; Segarra *et al.*, 2015), which would correspond to a reduction in emissions between 2% and 50%, assuming a source strength of approximately 200 Tg of methane per year (Fig. 2.1). A global estimation for peatlands resulted also in a 50% reduction of global methane emissions due to anaerobic methane oxidation, corresponding to 41 Tg of methane per year that are anaerobically oxidized (Smemo and Yavitt, 2011). Another study reported an average of 24 Tg of methane consumption per year by anaerobic methanotrophs in peatlands, with very high variation between sites (Gupta *et al.*, 2012). Based on these first estimates, it appears that anaerobic methane oxidation contributes significantly to a reduction in methane emissions in wetlands and should therefore be considered as a relevant process.

In marine ecosystems, anaerobic methane oxidation is usually coupled with sulfate reduction and mediated by anaerobic methanotrophic archaea, which are often found in a consortium with sulfate-reducing bacteria (Knittel and Boetius, 2009). These anaerobic methane-oxidizing archaea are *Euryarchaeota* that are phylogenetically related to methanogenic archaea and referred to as ANME clusters (Fig. 2.2). In contrast, microorganisms performing anaerobic methane oxidation coupled with denitrification are more common in continental ecosystems. Two major groups of microorganisms have been characterized that are involved in this process, represented by '*Candidatus* Methylomirabilis oxyfera' and '*Candidatus* Methanoperedens nitroreducens' (Ettwig *et al.*, 2010; Haroon *et al.*, 2013). Moreover, a methanotrophic archaeon coupling anaerobic methane oxidation with iron reduction '*Candidatus* Methanoperedens ferrireducens' has been described (Cai *et al.*, 2018). All these different anaerobic methanotrophs can be successfully enriched in bioreactors despite their slow growth rates, but pure cultures are not available.

Characteristics of the different anaerobic methanotrophs have been summarized in diverse review articles (e.g. Chistoserdova, 2015; Cui *et al.*, 2015; Kallistova *et al.*, 2017), some of them with a focus on methanotrophs that oxidize methane coupled with sulfate reduction (Knittel and Boetius, 2009) and others with a focus on those that couple methane oxidation with denitrification (Shen *et al.*, 2015a; Welte *et al.*, 2016). Yet others discuss in detail the

particular physiology of these organisms (Caldwell *et al.*, 2008; McGlynn, 2017; Timmers *et al.*, 2017). Within this review, relevant basic information about these organisms is given, but the focus will be on aspects related to their occurrence in soils.

Anaerobic methane oxidation coupled with sulfate reduction

Diversity and physiology of anaerobic methane oxidizers dependent on sulfate reduction

Three phylogenetic groups of anaerobic methane-oxidizing archaea are known that couple methane oxidation with sulfate reduction, ANME-1 to -3 (Knittel and Boetius, 2009; Timmers *et al.*, 2017). They were defined based on distinct clustering of their 16S rRNA and *mcrA* gene sequences in phylogenetic trees. The ANME-1 group has been reported to be distantly related to the *Methanosarcinales* and *Methanomicrobiales*. It now appears that they are also related to the recently described *Methanonatronarchaeales* (Fig. 2.2). The ANME-2 group is related to the *Methanosarcinales* and ANME-3 to the genus *Methanococcoides* within the order *Methanosarcinales*.

The ANME-1 group consists of subgroups a and b, ANME-2 of subgroups a, b and c. The groups ANME-2a and -2b form a coherent clade and are often grouped together as ANME-2a/b. Furthermore, an ANME-2d group has been proposed to exist, including '*Candidatus* Methanoperedens nitroreducens', which couples methane oxidation with denitrification (Haroon *et al.*, 2013). Earlier, this group was referred to as AOM associated archaea (AAA) group (Knittel and Boetius, 2009). In the literature, the name ANME-2d had been introduced once before for the related GOM Arc I sequence cluster, but the name was replaced by GOM Arc I because of missing evidence for anaerobic methane oxidation potential (Lloyd *et al.*, 2006). Today, the two clusters are sometimes combined again into a larger ANME–2d cluster, despite the missing evidence for methanotrophy in the GOM Arc I group, and this larger ANME-2d cluster is meanwhile divided into three subgroups based on 16S rRNA gene sequences (Welte *et al.*, 2016).

The ANME-1 and ANME-2 groups show a wide distribution in diverse marine environments, while ANME-3 has been predominantly detected in submarine mud volcanoes and marine methane seeps, indicating ecological niche separation (Knittel and Boetius, 2009; Cui *et al.*, 2015; Timmers *et al.*, 2017). The ANME organisms are frequently found in association with sulfate-reducing *Deltaproteobacteria*, ANME-1 and -2 with members of the *Desulfosarcina-Desulfococcus* group and ANME-3 preferentially with *Desulfobulbus* (Knittel and Boetius, 2009; Cui *et al.*, 2015). While the ANME organisms are performing methane oxidation to carbon dioxide, the sulfate reducers are responsible for sulfate reduction. Therefore, reducing equivalents are channelled between the two partners (McGlynn *et al.*, 2015; Wegener *et al.*, 2015). However, sometimes ANME groups have also been found in association with other bacterial taxa, and an association between sulfate reducers and ANME-1 is not consistently observed (Knittel and Boetius, 2009; Timmers *et al.*, 2017). This suggests that some ANME groups may perform the complete process alone, as proposed for ANME-2 organisms (Milucka *et al.*, 2012). Alternatively, they may couple methane oxidation to other not yet known reduction processes, either in association with a bacterial partner or possibly even alone. Moreover, it has been discussed that ANME-1 and ANME-2 organisms can perform methanogenesis instead of methane oxidation (House *et al.*, 2009; Bertram *et al.*, 2013). This metabolic versatility is the result of a genetic makeup that allows reverse methanogenesis for methane oxidation (Hallam *et al.*, 2004; Scheller *et al.*, 2010), but which can obviously still operate in the direction known from methanogenic archaea. Thus, the presence of ANME organisms can be linked to both, methane oxidation as well as methane production activity. The relative importance of a methanogenic activity needs to be studied in more detail in the future.

Evidence for anaerobic methane oxidation coupled to sulfate reduction in terrestrial ecosystems and soils

In continental ecosystems, anaerobic methane oxidation coupled with sulfate reduction is mostly considered to be of minor relevance, because sulfate concentrations are usually much lower than in marine ecosystems, rendering this process thermodynamically unfavourable (Smemo and Yavitt, 2011). However, internal redox-cycling of sulfur

compounds, e.g. due to fluctuating water levels, might support this type of methane oxidation even in continental ecosystems at lower sulfate concentrations. Indirect evidence from mass balance approaches and measurements of methane oxidation rates in combination with sulfate reduction rates points to the existence of this process, e.g. in natural wetlands, rice paddies and groundwater at a landfill leachate plume (Murase and Kimura, 1994; Grossman *et al.*, 2002; Segarra *et al.*, 2015). However, final proof for a coupling of anaerobic methane oxidation with sulfate reduction is not given in most of these studies and oxidation rates were often considered to be quantitatively unimportant. To further validate the existence of this process in continental ecosystems, the presence of anaerobic methane-oxidizing microorganisms and their active involvement in the methane oxidation process need to be carefully proven (Timmers *et al.*, 2016).

The detection of anaerobic methanotrophic archaea of the clusters ANME-1, -2 and -3 was initially limited to anoxic, methane-rich, sulfate-containing marine sediments (Knittel *et al.*, 2005). In the meantime, 16S rRNA gene sequences of ANME-1 and -2, especially those of the ANME-1a and -2a sub-clusters, have repeatedly been detected in some specific continental environments such as freshwater subsurfaces and methane seeps, oilfield production waters and mud volcanoes (e.g. Knittel and Boetius, 2009; Niederberger *et al.*, 2010; Chang *et al.*, 2012). Moreover, their presence has been shown in the terrestrial subsurface, in soils from natural gas fields and a eutrophic freshwater lake (Eller *et al.*, 2005; Fry *et al.*, 2009; Miyashita *et al.*, 2009). As presence does not necessarily imply activity, anaerobic methane oxidation was demonstrated to be an active process in freshwater sediment samples based on 16S rRNA recovery and methane oxidation rate measurements, which were shown to be stimulated by sulfate amendments (Takeuchi *et al.*, 2011; Timmers *et al.*, 2016). The strongest evidence for a coupling of anaerobic methane oxidation with sulfate reduction was provided in the study by Timmers *et al.* (2016), who evaluated methane oxidation and sulfate reduction activity and detected ANME-2a/b sequences along with sequences of sulfate reducers in freshwater sediment samples. In contrast, no sulfate reducers were identified in the studies of Takeuchi *et al.* (2011), where ANME 1 sequences were found in the freshwater subsurface, and of Chang *et al.* (2012), who detected ANME-1a and -2a in a mud volcano and proposed a coupling to metal reduction rather than to sulfate. A detection of (active) ANME organisms in soils from natural wetlands or rice paddies remained usually unsuccessful (Miyashita *et al.*, 2009), indicating that the occurrence of ANME organisms in continental environments is largely limited to some freshwater systems and particular habitats such as mud volcanoes.

Anaerobic methane oxidation coupled with denitrification

The coupling of anaerobic methane oxidation with denitrification was first detected in an enrichment culture obtained from an anoxic freshwater sediment rich in nitrate (Raghoebarsing *et al.*, 2006). The microorganisms being responsible for this process were identified as bacteria of the candidate phylum NC10 and referred to as '*Ca.* Methylomirabilis oxyfera' (Ettwig *et al.*, 2010). These so-called 'NC10 bacteria' couple the oxidation of methane with nitrite reduction to dinitrogen.

A few years after the discovery of '*Ca.* Methylomirabilis oxyfera' it turned out that anoxic incubations with methane and nitrate (and nitrite or ammonium in addition) for the enrichment of denitrifying anaerobic methanotrophic bacteria support the establishment of another anaerobic methanotroph, the archaeal '*Ca.* Methanoperedens nitroreducens', representing the ANME-2d group (Haroon *et al.*, 2013). This organism is a member of the order *Methanosarcinales*, class '*Candidatus* Methanoperedenaceae'. It couples methane oxidation with nitrate reduction to nitrite. Depending on the nitrogen sources that are provided, '*Ca.* Methanoperedens nitroreducens' can form syntrophic associations either with '*Ca.* Methanoperedens nitroreducens' or with anaerobic ammonium-oxidizing (anammox) bacteria (Raghoebarsing *et al.*, 2006; Haroon *et al.*, 2013; Arshad *et al.*, 2015; Vaksmaa *et al.*, 2017a; Gambelli *et al.*, 2018). It is assumed that '*Ca.* Methylomirabilis oxyfera' or the anammox bacteria support the growth of '*Ca.* Methanoperedens nitroreducens' by eliminating nitrite, which is toxic at high concentrations (Welte *et al.*, 2016).

Physiology of '*Ca.* Methylomirabilis oxyfera' and '*Ca.* Methanoperedens nitroreducens'

Physiological properties of '*Ca*. Methanoperedens nitroreducens' were derived from reconstructed genome information, which is meanwhile available for a couple of enrichment cultures (Haroon *et al.*, 2013; Arshad *et al.*, 2015; Berger *et al.*, 2017; Vaksmaa *et al.*, 2017a). This indicates the coupling of nitrate reduction to nitrite with reverse methanogenesis. Nitrate reduction is performed by a membrane-bound nitrate reductase that appears to be of bacterial origin, possibly obtained via horizontal gene transfer. A membrane-bound nitrite reductase may be involved in the conversion of nitrite to ammonium, which may contribute to the elimination of nitrite.

Similarly as for '*Ca*. Methanoperedens nitroreducens', metagenomic sequencing helped to get insight into the metabolism of '*Ca.* Methylomirabilis oxyfera'. These organisms have established a completely different mechanism to couple methane oxidation with nitrite reduction to dinitrogen. A methane oxidation pathway as known from aerobic methanotrophic bacteria is present, while reverse methanogenesis does not play a role. The aerobic methane oxidation pathway includes methane oxidation via a particulate methane monooxygenase, which demands oxygen. It is assumed that this oxygen is derived from an intra-aerobic pathway, in which dinitrogen and oxygen are obtained by a dismutation reaction of two molecules of nitric oxide (Ettwig *et al.*, 2010, 2012). Oxygen that is not used for methane oxidation may be reduced by a terminal oxidase, allowing to gain additional energy by oxygen respiration (Wu *et al.*, 2011). Despite the need for oxygen, '*Ca.* Methylomirabilis oxyfera' is an anaerobic organism. In the presence of ≥2% oxygen, methane oxidation and nitrite reduction activity decreased substantially and the cells encountered oxidative stress (Luesken *et al.*, 2012). However, it remains currently unclear whether they profit from external oxygen when available in trace amounts.

Diversity and environmental distribution of '*Ca.* Methylomirabilis oxyfera' and '*Ca.* Methanoperedens nitroreducens'

Besides '*Ca.* Methylomirabilis oxyfera', a second species of this candidate genus has been proposed in the meantime, '*Candidatus* Methylomirabilis sinica', enriched from paddy soil (He *et al.*, 2016). Several further reports describe the enrichment of bacteria coupling anaerobic methane oxidation with denitrification from diverse environmental samples, including paddy soils, peatland, river sediments, coastal sediments, wastewater and bioreactor sludges (Zhu *et al.*, 2012; He *et al.*, 2015; Bhattacharjee *et al.*, 2016; Chen *et al.*, 2016; Welte *et al.*, 2016; Vaksmaa *et al.*, 2017a). Furthermore, bacteria of the NC10 phylum were identified in several cultivation-independent studies (Shen *et al.*, 2015d; Chen *et al.*, 2016; Welte *et al.*, 2016). Such studies use the 16S rRNA or the *pmoA* gene as marker. Data interpretation of 16S rRNA gene marker based results has to be done with care, because the NC10 phylum includes four different 16S rRNA gene sequence clusters, but it is not yet clear whether all clusters represent anaerobic methanotrophic bacteria (Welte *et al.*, 2016). The use of *pmoA* as marker demands specific *pmoA* primers due to the distinct clustering of their *pmoA* sequences in phylogenetic trees (Fig. 2.4), and several different *pmoA* primer sets have been developed (Shen *et al.*, 2015d; Chen *et al.*, 2016). Analyses based on the 16S rRNA and *pmoA* marker genes indicate that methanotrophs of the NC10 phylum are present in diverse environments. The detection was successful in freshwater lake and river sediments, in different wetlands including peatlands and swamps, in paddy soils and a few times in other agricultural soils, in wastewater systems, in some marine and coastal sediments, and recently for the first time in the rumen fluid from goats (Chen *et al.*, 2016; Shen *et al.*, 2016b; Welte *et al.*, 2016; L. Liu *et al.*, 2017).

This broad occurrence is in line with predictions stating that anaerobic methane oxidation coupled to denitrification should be relevant in ecosystems with methane supply from anoxic compartments and availability of oxidized nitrogenous compounds (Thauer and Shima, 2008). Such conditions are found, for example, in wastewater treatment systems or near the oxic/anoxic interphase in paddy soils or freshwater ecosystems, especially when located in agricultural landscapes with high nitrogen input. Indeed, '*Ca.* Methylomirabilis oxyfera' can be detected at such oxic/anoxic interfaces in various wetlands (Raghoebarsing *et al.*, 2006; Zhu *et al.*, 2015). However, several studies

report the predominant occurrence in deeper layers of wetlands and paddy soils (Zhu *et al.*, 2012; Hu *et al.*, 2014; Shen *et al.*, 2015b,c), where anoxic conditions are more stable. Moreover, NC10 methanotrophs were more abundant in freshwater samples such as reservoir and pond sediments with rather stable anoxic conditions than in wetland sediments or paddy soils (Shen *et al.*, 2016b). However, if '*Ca.* Methylomirabilis oxyfera' colonizes indeed preferably habitats that provide stable anoxic conditions, the detection in upland soils is surprising, as reported for tropical forest samples in 5–20 cm depth (Meng *et al.*, 2016) and agricultural soil samples in 50–60 cm depth (Hu and Ma, 2016; Shen *et al.*, 2016a). However, a detection in upland soils was not consistently observed (Zhu *et al.*, 2015). The relevance of '*Ca.* Methylomirabilis oxyfera' in such soils remains currently completely unclear.

Similarly as '*Ca.* Methylomirabilis oxyfera', '*Ca.* Methanoperedens nitroreducens', shows a broad occurrence. It has been detected in lake and river sediments, aquifers, paddy soils, peatlands, mud volcanoes, sewage treatment plants and, to limited extent, in the marine and brackish environment (Ding *et al.*, 2015; Welte *et al.*, 2016; Narrowe *et al.*, 2017). The cultivation-independent detection is done using group-specific 16S rRNA gene primers or *mcrA* gene primers (Ding *et al.*, 2015; Vaksmaa *et al.*, 2017b; Xu *et al.*, 2018). The obtained 16S rRNA gene sequences can be classified into three different subgroups, which are not all represented by enrichment cultures, so that the methane oxidation potential of some sequence clusters remains currently unclear (Welte *et al.*, 2016).

As presence does not necessarily imply activity, studies are needed to assess the activity of these methanotrophs in the different ecosystems. This has so far only been done in a few studies, either at the transcript (Padilla *et al.*, 2016) or protein level (Hanson and Madsen, 2015) in a marine and freshwater environment, respectively, demonstrating metabolic activity of '*Ca.* Methylomirabilis oxyfera'. Some other studies demonstrated by isotope experiments that an anoxic conversion of methane in the presence of nitrite or nitrate as electron acceptor is occurring in wetlands, lake sediment and a rice paddy soil. These analyses were combined with molecular approaches, confirming the presence of '*Ca.* Methylomirabilis oxyfera' (Deutzmann and Schink, 2011; Hu *et al.*, 2014; Shen *et al.*, 2014, 2015c) and '*Ca.* Methanoperedens nitroreducens' (Vaksmaa *et al.*, 2016) in the corresponding samples. In the paddy soil study the determined methane uptake rates were substantially higher than reported in an earlier study, indicating that anaerobic methane oxidation may play a significant role in these soils (Vaksmaa *et al.*, 2016). Differences concerning the activity of these methanotrophs were also reported for natural wetlands, i.e. an activity of anaerobic methanotrophs could not always be proven (Tveit *et al.*, 2013). In general, the potential contribution of anaerobic denitrifying methanotrophs in carbon and nitrogen cycling needs to be studied in more detail in the diverse environments to assess their relevance more precisely.

Alternative electron acceptors for anaerobic methane oxidation

Besides sulfate, nitrate and nitrite, further electron acceptors have been discussed to be of relevance in combination with anaerobic methane oxidation. A coupling with iron reduction has been demonstrated in '*Candidatus* Methanoperedens ferrireducens' (Cai *et al.*, 2018). This archaeon was enriched in a bioreactor fed with methane and ferrihydrite, which was set up with material from a freshwater reservoir. Metagenomic analysis predicts that methane oxidation occurs via reverse methanogenesis and iron reduction by multihaem c-type cytochromes. The exact mechanism of the electron transfer to iron remains currently unclear. Earlier, a coupling of methane oxidation to iron reduction was already reported for an enrichment culture of '*Candidatus* Methanoperedens nitroreducens MPEBLZ', obtained from a freshwater sample and cultured in a reactor with methane and nitrate as substrates (Ettwig *et al.*, 2016). This culture was able to couple methane oxidation with nitrate or iron, but the rate of iron-based oxidation was 10-fold lower than in '*Ca.* Methanoperedens ferrireducens' (Cai *et al.*, 2018). Besides the use of nitrate and iron, '*Ca.* Methanoperedens nitroreducens MPEBLZ' was shown to reduce manganese. Obviously, '*Ca.* Methanoperedens' shows versatility concerning the use of electron acceptors, but may have substrate preferences. It remains currently unclear how commonly iron-reduction occurs within this candidate genus and how versatile the individual members are indeed.

The coupling of anaerobic methane oxidation with iron reduction has been demonstrated in incubation experiments with deep sea sediment material harbouring ANME-2c organisms (Scheller *et al.*, 2016) and by geochemical profiling or isotope tracer studies in environmental samples from lake water and sediment, marine sediments, paddy fields, a terrestrial mud volcano and a contaminated aquifer (Zandt *et al.*, 2018). The detection of the process and the presence of '*Ca.* Methanoperedens' species in such environments (Welte *et al.*, 2016; Narrowe *et al.*, 2017) suggests a high ecological relevance, especially in marine and freshwater ecosystems, where iron oxides are present in the sediments (Cai *et al.*, 2018), but more studies are needed that link the geochemical processes with microbiological data to demonstrate this relevance and identify the involved microorganisms.

In peatlands, methane oxidation coupled with iron reduction has also been discussed as an option because of low concentrations of available sulfate and nitrate, but could not yet be proven (Smemo and Yavitt, 2007, 2011; Gupta *et al.*, 2012). Alternatively, humic substances are considered to be of possible relevance, knowing that ANME organisms can transfer electrons to external acceptors (Scheller *et al.*, 2016) and that humic substances can act as electron acceptors (Scott *et al.*, 1998). Further evidence is provided by the observations that humic substances accumulate in peatlands and anaerobic methane oxidation was detected, but without strong evidence for a coupling with nitrate, sulfate or iron reduction (Smemo and Yavitt, 2007; Gupta *et al.*, 2012). Such a weak coupling with the known inorganic electron acceptors was also reported by Reed *et al.* (2017) for an eutrophic reservoir, along with the suggestion that organic acids, which are major constituents of organic matter, may serve as electron acceptors during anaerobic methane oxidation in eutrophic lakes and reservoirs.

These findings indicate that the geochemical characteristics of an ecosystem will have a major impact on the overall rate of anaerobic methane oxidation and the dominant type of methane oxidation process taking place. This is exemplified by the observation that anaerobic methane oxidation activity was quantitatively more important in nutrient-rich (minerotrophic) fens than in nutrient-poor (ombrotrophic) bogs (Smemo and Yavitt, 2007; Gupta *et al.*, 2012). Furthermore, Segarra *et al.* (2015) observed different dependencies on electron acceptors in different wetland systems. It can thus be concluded that anaerobic methane oxidation is of relevance in diverse terrestrial ecosystems. The coupling of anaerobic methane oxidation with specific reduction processes and the identity of the involved microorganisms appears to be ecosystem specific and requests a good understanding of the geochemical processes in combination with the physiology of the microorganisms inhabiting the respective ecosystems.

Conclusions and future perspectives

Microbiological processes leading to methane production and consumption are major drivers of the global methane cycle. For a long time, methane production was attributed to the activity of a few orders of methanogenic *Euryarchaeota*, while methane oxidation activity was ascribed to specific families of methanotrophic *Alpha-* and *Gammaproteobacteria*. Research of the last two decades has substantially extended the list of players in both groups. As described in this review, a couple of new orders of methanogens within the *Euryarchaeota* were discovered during the last years and metagenomic analyses suggest that methanogens may even exist beyond the phylum *Euryarchaeota*. Likewise, the methanotrophic lifestyle appears to be more widespread among microorganisms within the *Alpha-* and *Gammaproteobacteria* but also among other taxa, exemplified by the discovery of methanotrophy in the phylum *Verrucomicrobia*. Work of the last years also indicates that anaerobic methane oxidation plays an important role not only in marine ecosystems, but also in continental environments including natural as well as anthropogenic wetlands, which are known to represent major sources of atmospheric methane. Distinct groups of methanotrophs such as '*Ca.* Methylomirabilis' and '*Ca.* Methanoperedens' appear to be the major types responsible for this process in terrestrial ecosystems. In order to better understand the methane sink or source capacity of an ecosystem and the variation of emissions or uptake in space and over time, the activities of all groups involved in methane cycling need to be considered. Thus, there is a clear need for studies that integrate the activities of all players.

The spatial distribution, abundance and activity of the individual players is controlled by diverse environmental factors. Recent findings indicate that more controls need to be considered. Several of the recently discovered groups of methanogens and methanotrophs but also some of the well-known players show a broader metabolic versatility than previously thought. Thus their presence alone or their general metabolic activity does not necessarily allow direct conclusions about their involvement in methane cycling under given conditions. The methane oxidation activity of facultative or mixotrophic methanotrophs may for example be suppressed or modulated dependent on the availability of alternative carbon and energy sources such as acetate or dihydrogen. Similarly, the activity of anaerobic methanotrophs is largely dependent on the availability of suitable electron acceptors. This in turn can depend on the activities of other groups of organisms. Nitrite availability for '*Ca.* Methylomirabilis' will for example depend on the organisms that produce nitrite, e.g. aerobic nitrifiers in nearby oxic habitats or '*Ca.* Methanoperedens' in the same anoxic habitat, as well as on the presence and activity of potential competitors such as anammox bacteria. A good understanding of such dependencies helps to define the ecological niche of these bacteria in natural as well as in artificial ecosystems such as wastewater treatment systems, where they could be introduced to reduce methane emissions (van Kessel *et al.*, 2018).

This review also demonstrates that the control of methanotrophic activity by specific environmental factors has to be assessed at a finer scale, at least for some factors. Exemplarily, the dependency on oxygen is highlighted, due to the fact that recent studies revealed interesting new insights. On the one hand, several methanogenic archaea appear to be less sensitive to oxygen than previously thought and methane production may even occur under apparently oxic conditions, on the other hand, some aerobic methanotrophic bacteria seem to be less dependent on oxygen and may remain active under apparently anoxic conditions. Considering that anaerobic methane oxidation is a process that occurs in terrestrial ecosystems such as wetlands, the general and rather simple assumption that oxic conditions support methane oxidation activity and anoxic conditions methane production appears to be too simple. Such general assumptions are still used in many models (Gauthier *et al.*, 2015). Besides an improvement of current models, the effect of oxygen on the distribution and activity of aerobic methanotrophs needs to be studied in more detail in soils. It has so far mostly been analysed in well-stratified ecosystems, especially lakes, but may be of equal relevance for the control of methanotrophs and methanogens in wetland soils, which show a higher variation in oxygen availability in space and over time.

As evident from this review, work of the last years has substantially extended the known diversity of methanotrophic and methanogenic microorganisms as well as knowledge about their metabolic capabilities. The discovery of new putative methanogenic and methanotrophic organisms in taxonomic groups not yet known to include such organisms was mostly the result of genome sequencing efforts. Physiological capabilities of uncultured organisms can now be derived quite easily from such genome reconstructions. Thousands of genomes are meanwhile available (e.g. Whitman *et al.*, 2015; Anantharaman *et al.*, 2016; Parks *et al.*, 2017) and many more datasets can be expected to be generated in the near future, either by *de novo* sequencing of isolates, single-cell based approaches for the analysis of uncultivated bacteria or assembly of genomes from metagenomic data. The analysis of these data will provide further insight into the metabolic versatility of known methane cycling microorganisms and very likely lead to the identification of further groups of microorganisms harbouring methane cycling potential in phylogenetic groups that are not yet known to include methanogens or methanotrophs. It will be a major task to validate the methane production or oxidation capabilities of these organisms. Some of these microorganisms may have a broader metabolic versatility compared with the canonical methanotrophs and methanogens, so that their activities and therewith their contribution to methane cycling in an ecosystem needs to be assessed carefully, e.g. by applying multi-omics approaches including metatransriptomics, -proteomics and possibly -metabolomics. Such studies should be complemented by laboratory analyses of microcosms, enrichment cultures or isolates under controlled conditions to identify and to understand the regulatory mechanisms that determine methanogenic or methanotrophic activity rates of

the different players. Genomic information available for uncultivated microorganisms may help to enrich and isolate these organisms, making them accessible for in-depth studies.

References

Ahn, J.H., Choi, M.Y., Kim, B.Y., Lee, J.S., Song, J., Kim, G.Y., and Weon, H.Y. (2014). Effects of water-saving irrigation on emissions of greenhouse gases and prokaryotic communities in rice paddy soil. Microb. Ecol. *68*, 271–283. https://doi.org/10.1007/s00248-014-0371-z

Anantharaman, K., Brown, C.T., Hug, L.A., Sharon, I., Castelle, C.J., Probst, A.J., Thomas, B.C., Singh, A., Wilkins, M.J., Karaoz, U., *et al.* (2016). Thousands of microbial genomes shed light on interconnected biogeochemical processes in an aquifer system. Nat. Commun. *7*, 13219. https://doi.org/10.1038/ncomms13219

Angel, R., Matthies, D., and Conrad, R. (2011). Activation of methanogenesis in arid biological soil crusts despite the presence of oxygen. PLOS ONE *6*, e20453. https://doi.org/10.1371/journal.pone.0020453

Angel, R., Claus, P., and Conrad, R. (2012). Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. ISME J. *6*, 847–862. https://doi.org/10.1038/ismej.2011.141

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

Aronson, E.L., Allison, S.D., and Helliker, B.R. (2013). Environmental impacts on the diversity of methane-cycling microbes and their resultant function. Front. Microbiol. *4*, 225. https://doi.org/10.3389/fmicb.2013.00225

Arshad, A., Speth, D.R., de Graaf, R.M., Op den Camp, H.J., Jetten, M.S., and Welte, C.U. (2015). A metagenomics-based metabolic model of nitrate-dependent anaerobic oxidation of methane by *Methanoperedens*-like archaea. Front. Microbiol. *6*, 1423. https://doi.org/10.3389/fmicb.2015.01423

Aschenbach, K., Conrad, R., Reháková, K., Doležal, J., Janatková, K., and Angel, R. (2013). Methanogens at the top of the world: occurrence and potential activity of methanogens in newly deglaciated soils in high-altitude cold deserts in the Western Himalayas. Front. Microbiol. *4*, 359. https://doi.org/10.3389/fmicb.2013.00359

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

Bender, M., and Conrad, R. (1992). Kinetics of CH_4 oxidation in oxic soils exposed to ambient air or high CH_4 mixing ratios. FEMS Microbiol. Ecol. *101*, 261–270. https://doi.org/10.1111/j.1574-6968.1992.tb05783.x

Berger, S., Frank, J., Dalcin Martins, P., Jetten, M.S.M., and Welte, C.U. (2017). High-quality draft genome sequence of '*Candidatus* Methanoperedens sp.' Strain BLZ2, a nitrate-reducing anaerobic methane-oxidizing archaeon enriched in an anoxic bioreactor. Genome Announc. *5*, e01159–17.

Bertram, S., Blumenberg, M., Michaelis, W., Siegert, M., Krüger, M., and Seifert, R. (2013). Methanogenic capabilities of ANME-archaea deduced from ^{13}C-labelling approaches. Environ. Microbiol. *15*, 2384–2393. https://doi.org/10.1111/1462-2920.12112

Bhattacharjee, A.S., Motlagh, A.M., Jetten, M.S.M., and Goel, R. (2016). Methane dependent denitrification-from ecosystem to laboratory-scale enrichment for engineering applications. Water Res. *99*, 244–252.

Biderre-Petit, C., Jézéquel, D., Dugat-Bony, E., Lopes, F., Kuever, J., Borrel, G., Viollier, E., Fonty, G., and Peyret, P. (2011). Identification of microbial communities involved in the methane cycle of a freshwater meromictic lake. FEMS Microbiol. Ecol. *77*, 533–545. https://doi.org/10.1111/j.1574-6941.2011.01134.x

Blees, J., Niemann, H., Wenk, C.B., Zopfi, J., Schubert, C.J., Kirf, M.K., Veronesi, M.L., Hitz, C., and Lehmann, M.F. (2014). Micro-aerobic bacterial methane oxidation in the chemocline and anoxic water column of deep south-Alpine Lake Lugano (Switzerland). Limnol. Oceanogr. *59*, 311–324. https://doi.org/10.4319/lo.2014.59.2.0311

Bogard, M.J., del Giorgio, P.A., Boutet, L., Chaves, M.C., Prairie, Y.T., Merante, A., and Derry, A.M. (2014). Oxic water column methanogenesis as a major component of aquatic CH_4 fluxes. Nat. Commun. *5*, 5350. https://doi.org/10.1038/ncomms6350

Borrel, G., Harriss, H.M.B., Parisot, N., Gaci, N., Tottey, W., Mihajlovski, A., Deane, J., Gribaldo, S., Bardot, O., Peyretaillade, E., *et al.* (2013a). Genome sequence of *'Candidatus* Methanomassiliicoccus intestinalis' Issoire-Mx1, a third *Thermoplasmatales*-related methanogenic archaeon from human feces. Genome Announc. *1*, e00453-00413. https://doi.org/10.1128/genomeA.00453-13

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

Borrel, G., McCann, A., Deane, J., Neto, M.C., Lynch, D.B., Brugère, J.F., and O'Toole, P.W. (2017). Genomics and metagenomics of trimethylamine-utilizing *Archaea* in the human gut microbiome. ISME J. *11*, 2059–2074. https://doi.org/10.1038/ismej.2017.72

Cai, C., Leu, A.O., Xie, G.J., Guo, J., Feng, Y., Zhao, J.X., Tyson, G.W., Yuan, Z., and Hu, S. (2018). A methanotrophic archaeon couples anaerobic oxidation of methane to Fe(III) reduction. ISME J. *12*, 1929–1939. https://doi.org/10.1038/s41396-018-0109-x

Caldwell, S.L., Laidler, J.R., Brewer, E.A., Eberly, J.O., Sandborgh, S.C., and Colwell, F.S. (2008). Anaerobic oxidation of methane: mechanisms, bioenergetics, and the ecology of associated microorganisms. Environ. Sci. Technol. *42*, 6791–6799.

Campbell, M.A., Nyerges, G., Kozlowski, J.A., Poret-Peterson, A.T., Stein, L.Y., and Klotz, M.G. (2011). Model of the molecular basis for hydroxylamine oxidation and

nitrous oxide production in methanotrophic bacteria. FEMS Microbiol. Lett. *322*, 82–89. https://doi.org/10.1111/j.1574-6968.2011.02340.x

Carere, C.R., Hards, K., Houghton, K.M., Power, J.F., McDonald, B., Collet, C., Gapes, D.J., Sparling, R., Boyd, E.S., Cook, G.M., *et al.* (2017). Mixotrophy drives niche expansion of verrucomicrobial methanotrophs. ISME J. *11*, 2599–2610. https://doi.org/10.1038/ismej.2017.112

Chang, Y.H., Cheng, T.W., Lai, W.J., Tsai, W.Y., Sun, C.H., Lin, L.H., and Wang, P.L. (2012). Microbial methane cycling in a terrestrial mud volcano in eastern Taiwan. Environ. Microbiol. *14*, 895–908. https://doi.org/10.1111/j.1462-2920.2011.02658.x

Chen, J., Dick, R., Lin, J.G., and Gu, J.D. (2016). Current advances in molecular methods for detection of nitrite-dependent anaerobic methane oxidizing bacteria in natural environments. Appl. Microbiol. Biotechnol. *100*, 9845–9860. https://doi.org/10.1007/s00253-016-7853-5

Chen, Y.P., and Yoch, D.C. (1987). Regulation of two nickel-requiring (inducible and constitutive) hydrogenases and their coupling to nitrogenase in *Methylosinus trichosporium* OB3b. J. Bacteriol. *169*, 4778–4783.

Chistoserdova, L. (2015). Methylotrophs in natural habitats: current insights through metagenomics. Appl. Microbiol. Biotechnol. *99*, 5763–5779. https://doi.org/10.1007/s00253-015-6713-z

Chojnacka, A., Szczęsny, P., Błaszczyk, M.K., Zielenkiewicz, U., Detman, A., Salamon, A., and Sikora, A. (2015). Noteworthy facts about a methane-producing microbial community processing acidic effluent from sugar beet molasses fermentation. PLOS ONE *10*, e0128008. https://doi.org/10.1371/journal.pone.0128008

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, B., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., *et al.* (2013). Carbon and other biogeochemical cycles. In Climate Change 2013: The Physical Science Basis Contribution of Working Group I to the Fifth Assessment Report in the Intergovernmental Panel on Climate Change, T.F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P.M. Midgley, eds. (Cambridge University Press, Cambridge, UK, and New York, NY). https://doi.org/10.1017/CBO9781107415324.015

Conrad, R. (1995). Soil microbial processes involved in production and consumption of atmospheric trace gases. Adv. Microb. Ecol. *14*, 207–250. https://doi.org/10.1007/978-1-4684-7724-5_5

Conrad, R. (1996). Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, 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. https://doi.org/10.1111/j.1574-6941.1999.tb00575.x

Conrad, R. (2005). Quantification of methanogenic pathways using stable carbon isotopic signatures: a review and a proposal. Org. Geochem. *36*, 739–752. https://doi.org/10.1016/j.orggeochem.2004.09.006

Conrad, R. (2007). Microbial ecology of methanogens and methanotrophs. Adv. Agron. *96*, 1–63. https://doi.org/10.1016/S0065-2113(07)96005-8

Conrad, R. (2009). The global methane cycle: recent advances in understanding the microbial processes involved. Environ. Microbiol. Rep. *1*, 285–292. https://doi.org/10.1111/j.1758-2229.2009.00038.x

Conrad, R., Erkel, C., and Liesack, W. (2006). Rice Cluster I methanogens, an important group of *Archaea* producing greenhouse gas in soil. Curr. Opin. Biotechnol. *17*, 262–267.

Costa, K.C., and Leigh, J.A. (2014). Metabolic versatility in methanogens. Curr. Opin. Biotechnol. *29*, 70–75. https://doi.org/10.1016/j.copbio.2014.02.012

Crevecoeur, S., Vincent, W.F., Comte, J., and Lovejoy, C. (2015). Bacterial community structure across environmental gradients in permafrost thaw ponds: methanotroph-rich ecosystems. Front. Microbiol. *6*, 192. https://doi.org/10.3389/fmicb.2015.00192

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

Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., *et al.* (2015). Complete nitrification by *Nitrospira* bacteria. Nature *528*, 504–509. https://doi.org/10.1038/nature16461

Dalal, R.C., and Allen, D.E. (2008). Greenhouse gas fluxes from natural ecosystems. Austr. J. Bot. *56*, 369–407. https://doi.org/10.1071/bt07128

Dalal, R.C., Allen, D.E., Livesley, S.J., and 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. https://doi.org/10.1007/s11104-007-9446-7

Dam, B., Dam, S., Blom, J., and Liesack, W. (2013). Genome analysis coupled with physiological studies reveals a diverse nitrogen metabolism in *Methylocystis* sp. strain SC2. PLOS ONE *8*, e74767. https://doi.org/10.1371/journal.pone.0074767

Danilova, O.V., Belova, S.E., Gagarinova, I.V., and Dedysh, S.N. (2016a). Microbial community composition and methanotroph diversity of a subarctic wetland in Russia. Mikrobiologiia *85*, 545–554.

Danilova, O.V., Suzina, N.E., Van De Kamp, J., Svenning, M.M., Bodrossy, L., and Dedysh, S.N. (2016b). A new cell morphotype among methane oxidizers: a spiral-shaped obligately microaerophilic methanotroph from northern low-oxygen environments. ISME J. *10*, 2734–2743. https://doi.org/10.1038/ismej.2016.48

Dedysh, S.N., and Knief, C. (2018). Diversity and phylogeny of described aerobic methanotrophs. In Methane Biocatalysis: Paving the Way to Sustainability, M.G. Kalyuzhanya, and X.-H. Xing, eds. (Springer, Cham, Switzerland), pp. 17–42. https://doi.org/10.1007/978-3-319-74866-5_2

Deng, Y., Cui, X., and Dumont, M.G. (2016). Identification of active aerobic methanotrophs in plateau wetlands using DNA stable isotope probing. FEMS Microbiol. Lett. *363*, fnw168. https://doi.org/10.1093/femsle/fnw168

Deutzmann, J.S., and Schink, B. (2011). Anaerobic oxidation of methane in sediments of Lake Constance, an oligotrophic freshwater lake. Appl. Environ. Microbiol. *77*, 4429–4436. https://doi.org/10.1128/AEM.00340-11

Deutzmann, J.S., Hoppert, M., and Schink, B. (2014). Characterization and phylogeny of a novel methanotroph, *Methyloglobulus morosus* gen. nov., spec. nov. Syst. Appl. Microbiol. *37*, 165–169. https://doi.org/10.1016/j.syapm.2014.02.001

Ding, J., Ding, Z.W., Fu, L., Lu, Y.Z., Cheng, S.H., and Zeng, R.J. (2015). New primers for detecting and quantifying denitrifying anaerobic methane oxidation archaea in different ecological niches. Appl. Microbiol. Biotechnol. *99*, 9805–9812. https://doi.org/10.1007/s00253-015-6893-6896.

Dlugokencky, E.J. (2018). NOAA/ESRL L (www.esrl.noaa.gov/gmd/ccgg/trends_ch4/).

Dlugokencky, E.J., Nisbet, E.G., Fisher, R., and Lowry, D. (2011). Global atmospheric methane: budget, changes and dangers. Philos. Trans. A Math. Phys. Eng. Sci. *369*, 2058–2072. https://doi.org/10.1098/rsta.2010.0341

Donis, D., Flury, S., Stöckli, A., Spangenberg, J.E., Vachon, D., and McGinnis, D.F. (2017). Full-scale evaluation of methane production under oxic conditions in a mesotrophic lake. Nat. Commun. *8*, 1661. https://doi.org/10.1038/s41467-017-01648-4

Dridi, B., Fardeau, M.L., Ollivier, B., Raoult, D., and Drancourt, M. (2012). *Methanomassiliicoccus luminyensis* gen. nov., sp nov., a methanogenic archaeon isolated from human faeces. Int. J. Syst. Evol. Microbiol. *62*, 1902–1907. https://doi.org/10.1099/ijs.0.033712-0

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

Dunfield, P.F. (2007). The soil methane sink. In Greenhouse Gas Sinks, D.S. Reay, C.N. Hewitt, K.A. Smith, and J. Grace, eds. (CABI Wallingford, UK), pp. 152–170. https://doi.org/10.1079/9781845931896.0152

Eder, W., Schmidt, M., Koch, M., Garbe-Schönberg, D., and Huber, R. (2002). Prokaryotic phylogenetic diversity and corresponding geochemical data of the brine-seawater interface of the Shaban Deep, Red Sea. Environ. Microbiol. *4*, 758–763.

Edwards, C.R., Onstott, T.C., Miller, J.M., Wiggins, J.B., Wang, W., Lee, C.K., Cary, S.C., Pointing, S.B., and Lau, M.C.Y. (2017). Draft genome sequence of uncultured upland soil cluster *Gammaproteobacteria* gives molecular insights into high-affinity methanotrophy. Genome Announc. *5*, e00047–17.

Eller, G., Känel, L., and Krüger, M. (2005). Cooccurrence of aerobic and anaerobic methane oxidation in the water column of Lake Plusssee. Appl. Environ. Microbiol. *71*, 8925–8928.

Ettwig, K.F., Butler, M.K., Le Paslier, D., Pelletier, E., Mangenot, S., Kuypers, M.M., Schreiber, F., Dutilh, B.E., Zedelius, J., de Beer, D., *et al.* (2010). Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. Nature *464*, 543–548. https://doi.org/10.1038/nature08883

Ettwig, K.F., Speth, D.R., Reimann, J., Wu, M.L., Jetten, M.S., and Keltjens, J.T. (2012). Bacterial oxygen production in the dark. Front. Microbiol. *3*, 273. https://doi.org/10.3389/fmicb.2012.00273

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

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

Ferry, J.G. (2010). The chemical biology of methanogenesis. Planet Space Sci. *58*, 1775–1783. https://doi.org/10.1016/j.pss.2010.08.014

Fetzer, S., Bak, F., and Conrad, R. (1993). Sensitivity of methanogenic bacteria from paddy soil to oxygen and desiccation. FEMS Microbiol. Ecol. *12*, 107–115.

Frindte, K., Maarastawi, S.A., Lipski, A., Hamacher, J., and Knief, C. (2017). Characterization of the first rice paddy cluster I isolate, *Methyloterricola oryzae* gen. nov., sp. nov. and amended description of *Methylomagnum ishizawai*. Int. J. Syst. Evol. Microbiol. *67*, 4507–4514. https://doi.org/10.1099/ijsem.0.002319

Fry, J.C., Horsfield, B., Skyes, R., Cragg, B.A., Heywood, C., Kim, G.T., Mangelsdorf, K., Mildenhall, D.C., Rinna, J., Vieth, A., *et al.* (2009). Prokaryotic populations and activities in an interbedded coal deposit, including a previously deeply buried section (1.6 - 2.3 km) above ~ 150 Ma basement rock. Geomicrobiol. J. *26*, 163–178. https://doi.org/10.1080/01490450902724832

Gambelli, L., Guerrero-Cruz, S., Mesman, R.J., Cremers, G., Jetten, M.S.M., Op den Camp, H.J.M., Kartal, B., Lueke, C., and van Niftrik, L. (2018). Community composition and ultrastructure of a nitrate-dependent anaerobic methane-oxidizing enrichment culture. Appl. Environ. Microbiol. *84*, e02186–17.

Garcia, J.L., Patel, B.K., and Ollivier, B. (2000). Taxonomic, phylogenetic, and ecological diversity of methanogenic Archaea. Anaerobe *6*, 205–226.

Garrity, G.M., Labeda, D.P., and Oren, A. (2011). XIIth international (IUMS) congress of bacteriology and applied microbiology. Int. J. Syst. Evol. Microbiol. *61*, 2775–2780. https://doi.org/10.1099/ijs.0.037366-0

Gauthier, M., Bradley, R.L., and Simek, M. (2015). More evidence that anaerobic oxidation of methane is prevalent in soils: Is it time to upgrade our biogeochemical models? Soil Biol. Biochem. *80*, 167–174. https://doi.org/10.1016/j.soilbio.2014.10.009

Grossart, H.P., Frindte, K., Dziallas, C., Eckert, W., and Tang, K.W. (2011). Microbial methane production in oxygenated water column of an oligotrophic lake. Proc. Natl. Acad. Sci. U.S.A. *108*, 19657–19661. https://doi.org/10.1073/pnas.1110716108

Großkopf, R., Stubner, S., and Liesack, W. (1998). Novel euryarchaeotal lineages detected on rice roots and in the anoxic bulk soil of flooded rice microcosms. Appl. Environ. Microbiol. *64*, 4983–4989.

Grossman, E.L., Cifuentes, L.A., and Cozzarelli, I.M. (2002). Anaerobic methane oxidation in a landfill-leachate plume. Environ. Sci. Technol. *36*, 2436–2442.

Gu, Q., Chang, S.X., Wang, Z.P., Feng, J.C., Chen, Q.S., and Han, X.G. (2016). Microbial versus non-microbial methane releases from fresh soils at different temperatures. Geoderma *284*, 178–184. https://doi.org/10.1016/j.geoderma.2016.08.027

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

Hallam, S.J., Putnam, N., Preston, C.M., Detter, J.C., Rokhsar, D., Richardson, P.M., and DeLong, E.F. (2004). Reverse methanogenesis: testing the hypothesis with environmental genomics. Science *305*, 1457–1462. https://doi.org/10.1126/science.1100025

Hanczár, T., Csáki, R., Bodrossy, L., Murrell, J.C., and Kovács, K.L. (2002). Detection and localization of two hydrogenases in *Methylococcus capsulatus* (Bath) and their potential role in methane metabolism. Arch. Microbiol. *177*, 167–172. https://doi.org/10.1007/s00203-001-0372-4

Hanson, B.T., and Madsen, E.L. (2015). In situ expression of nitrite-dependent anaerobic methane oxidation proteins by *Candidatus* Methylomirabilis oxyfera co-occurring with expressed anammox proteins in a contaminated aquifer. Environ. Microbiol. Rep. *7*, 252–264. https://doi.org/10.1111/1758-2229.12239

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

He, Y., Li, M., Perumal, V., Feng, X., Fang, J., Xie, J., Sievert, S.M., and Wang, F. (2016). Genomic and enzymatic evidence for acetogenesis among multiple lineages of the archaeal phylum *Bathyarchaeota* widespread in marine sediments. Nat. Microbiol. *1*, 16035. https://doi.org/10.1038/nmicrobiol.2016.35

He, Z., Cai, C., Shen, L., Lou, L., Zheng, P., Xu, X., and Hu, B. (2015). Effect of inoculum sources on the enrichment of nitrite-dependent anaerobic methane-oxidizing bacteria. Appl. Microbiol. Biotechnol. *99*, 939–946. https://doi.org/10.1007/s00253-014-6033-8

He, Z., Cai, C., Wang, J., Xu, X., Zheng, P., Jetten, M.S., and Hu, B. (2016). A novel denitrifying methanotroph of the NC10 phylum and its microcolony. Sci. Rep. *6*, 32241. https://doi.org/10.1038/srep32241

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

Hernández, M., Conrad, R., Klose, M., Ma, K., and Lu, Y.H. (2017). Structure and function of methanogenic microbial communities in soils from flooded rice and upland soybean fields from Sanjiang plain, NE China. Soil Biol. Biochem. *105*, 81–91. https://doi.org/10.1016/j.soilbio.2016.11.010

Hofmann, K., Praeg, N., Mutschlechner, M., Wagner, A.O., and Illmer, P. (2016). Abundance and potential metabolic activity of methanogens in well-aerated forest and grassland soils of an alpine region. FEMS Microbiol. Ecol. *92*, fiv171. https://doi.org/10.1093/femsec/fiv171

House, C.H., Orphan, V.J., Turk, K.A., Thomas, B., Pernthaler, A., Vrentas, J.M., and Joye, S.B. (2009). Extensive carbon isotopic heterogeneity among methane seep microbiota. Environ. Microbiol. *11*, 2207–2215. https://doi.org/10.1111/j.1462-2920.2009.01934.x

Hu, B.L., Shen, L.D., Lian, X., Zhu, Q., Liu, S., Huang, Q., He, Z.F., Geng, S., Cheng, D.Q., Lou, L.P., *et al.* (2014). Evidence for nitrite-dependent anaerobic methane oxidation as a previously overlooked microbial methane sink in wetlands. Proc. Natl. Acad. Sci. U.S.A. *111*, 4495–4500. https://doi.org/10.1073/pnas.1318393111

Hu, H.W., Zhang, L.M., Yuan, C.L., and He, J.Z. (2013). Contrasting Euryarchaeota communities between upland and paddy soils exhibited similar pH-impacted biogeographic patterns. Soil Biol. Biochem. *64*, 18–27. https://doi.org/10.1016/j.soilbio.2013.04.003

Hu, Z., and Ma, R. (2016). Distribution and characteristic of nitrite-dependent anaerobic methane oxidation bacteria by comparative analysis of wastewater treatment plants and agriculture fields in northern China. PeerJ *4*, e2766. https://doi.org/10.7717/peerj.2766

Iino, T., Tamaki, H., Tamazawa, S., Ueno, Y., Ohkuma, M., Suzuki, K., Igarashi, Y., and Haruta, S. (2013). *Candidatus* Methanogranum caenicola: a novel methanogen from the anaerobic digested sludge, and proposal of *Methanomassiliicoccaceae* fam. nov. and *Methanomassiliicoccales* ord. nov., for a methanogenic lineage of the class *Thermoplasmata*. Microbes Environ. *28*, 244–250.

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

in 't Zandt, M.H., de Jong, A.E., Slomp, C.P., and Jetten, M.S. (2018). The hunt for the most-wanted chemolithoautotrophic spookmicrobes. FEMS Microbiol. Ecol. *94*. https://doi.org/10.1093/femsec/fiy064

Kallistova, A.Y., Merkel, A.Y., Tarnovetskii, I.Y., and Pimenov, N.V. (2017). Methane formation and oxidation by prokaryotes. Microbiology *86*, 671–691. https://doi.org/10.1134/S0026261717060091

Kalyuzhnaya, M.G., Yang, S., Rozova, O.N., Smalley, N.E., Clubb, J., Lamb, A., Gowda, G.A., Raftery, D., Fu, Y., Bringel, F., *et al.* (2013). Highly efficient methane biocatalysis revealed in a methanotrophic bacterium. Nat. Commun. *4*, 2785. https://doi.org/10.1038/ncomms3785

Kalyuzhnaya, M.G., Lamb, A.E., McTaggart, T.L., Oshkin, I.Y., Shapiro, N., Woyke, T., and Chistoserdova, L. (2015). Draft genome sequences of gammaproteobacterial methanotrophs isolated from Lake Washington sediment. Genome Announc. *3*, e00103–15. https://doi.org/10.1128/genomeA.00103-15

Kammann, C., Hepp, S., Lenhart, K., and Mulller, C. (2009). Stimulation of methane consumption by endogenous CH_4 production in aerobic grassland soil. Soil Biol.

Biochem. *41*, 622–629. https://doi.org/10.1016/j.soilbio.2008.12.025

Kammann, C., Ratering, S., Görres, C.M., Guillet, C., and Muller, C. (2017). Stimulation of methane oxidation by CH_4-emitting rose chafer larvae in well-aerated grassland soil. Biol. Fertil. Soils *53*, 491–499. https://doi.org/10.1007/s00374-017-1199-8

Kao-Kniffin, J., Woodcroft, B.J., Carver, S.M., Bockheim, J.G., Handelsman, J., Tyson, G.W., Hinkel, K.M., and Mueller, C.W. (2015). Archaeal and bacterial communities across a chronosequence of drained lake basins in Arctic Alaska. Sci. Rep. *5*, 18165. https://doi.org/10.1038/srep18165

Kirschke, S., Bousquet, P., Ciais, P., Saunois, M., Canadell, J.G., Dlugokencky, E.J., Bergamaschi, P., Bergmann, D., Blake, D.R., Bruhwiler, L., *et al.* (2013). Three decades of global methane sources and sinks. Nat. Geosci. *6*, 813–823. https://doi.org/10.1038/Ngeo1955

Kits, K.D., Campbell, D.J., Rosana, A.R., and Stein, L.Y. (2015a). Diverse electron sources support denitrification under hypoxia in the obligate methanotroph *Methylomicrobium album* strain BG8. Front. Microbiol. *6*, 1072. https://doi.org/10.3389/fmicb.2015.01072

Kits, K.D., Klotz, M.G., and Stein, L.Y. (2015b). Methane oxidation coupled to nitrate reduction under hypoxia by the Gammaproteobacterium *Methylomonas denitrificans*, sp. nov. type strain FJG1. Environ. Microbiol. *17*, 3219–3232. https://doi.org/10.1111/1462-2920.12772

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

Knief, C., Lipski, A., and Dunfield, P.F. (2003). Diversity and activity of methanotrophic bacteria in different upland soils. Appl. Environ. Microbiol. *69*, 6703–6714.

Knief, C., Delmotte, N., Chaffron, S., Stark, M., Innerebner, G., Wassmann, R., von Mering, C., and Vorholt, J.A. (2012). Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. ISME J. *6*, 1378–1390. https://doi.org/10.1038/ismej.2011.192

Knittel, K., and Boetius, A. (2009). Anaerobic oxidation of methane: progress with an unknown process. Annu. Rev. Microbiol. *63*, 311–334. https://doi.org/10.1146/annurev.micro.61.080706.093130

Knittel, K., Lösekann, T., Boetius, A., Kort, R., and Amann, R. (2005). Diversity and distribution of methanotrophic archaea at cold seeps. Appl. Environ. Microbiol. *71*, 467–479.

Kojima, H., Tokizawa, R., Kogure, K., Kobayashi, Y., Itoh, M., Shiah, F.K., Okuda, N., and Fukui, M. (2014). Community structure of planktonic methane-oxidizing bacteria in a subtropical reservoir characterized by dominance of phylotype closely related to nitrite reducer. Sci. Rep. *4*, 5728. https://doi.org/10.1038/srep05728

Kolb, S. (2009). The quest for atmospheric methane oxidizers in forest soils. Environ. Microbiol. Rep. *1*, 336–346. https://doi.org/10.1111/j.1758-2229.2009.00047.x

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

Lang, K., Schuldes, J., Klingl, A., Poehlein, A., Daniel, R., and Brunea, 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.

Laso-Pérez, R., Wegener, G., Knittel, K., Widdel, F., Harding, K.J., Krukenberg, V., Meier, D.V., Richter, M., Tegetmeyer, H.E., Riedel, D., *et al.* (2016). Thermophilic archaea activate butane via alkyl-coenzyme M formation. Nature *539*, 396–401. https://doi.org/10.1038/nature20152

Lazar, C.S., Baker, B.J., Seitz, K., Hyde, A.S., Dick, G.J., Hinrichs, K.U., and Teske, A.P. (2016). Genomic evidence for distinct carbon substrate preferences and ecological niches of *Bathyarchaeota* in estuarine sediments. Environ. Microbiol. *18*, 1200–1211. https://doi.org/10.1111/1462-2920.13142

Lee, C.G., Watanabe, T., Murase, J., Asakawa, S., and Kimura, M. (2012). Growth of methanogens in an oxic soil microcosm: Elucidation by a DNA-SIP experiment using ^{13}C-labeled dried rice callus. Appl. Soil Ecol. *58*, 37–44. https://doi.org/10.1016/j.apsoil.2012.03.002

Lee, H.J., Jeong, S.E., Kim, P.J., Madsen, E.L., and Jeon, C.O. (2015). High resolution depth distribution of *Bacteria*, *Archaea*, methanotrophs, and methanogens in the bulk and rhizosphere soils of a flooded rice paddy. Front. Microbiol. *6*, 639. https://doi.org/10.3389/fmicb.2015.00639

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

Li, W., Guan, W., Chen, H., Liao, B.W., Hu, J., Peng, C.H., Rui, J.P., Tian, J.Q., Zhu, D., and He, Y.X. (2016). Archaeal communities in the sediments of different mangrove stands at Dongzhaigang, China. J. Soils Sediments *16*, 1995–2004. https://doi.org/10.1007/s11368-016-1427-0

Li, Y., Leahy, S.C., Jeyanathan, J., Henderson, G., Cox, F., Altermann, E., Kelly, W.J., Lambie, S.C., Janssen, P.H., Rakonjac, J., *et al.* (2016). The complete genome sequence of the methanogenic archaeon ISO4-H5 provides insights into the methylotrophic lifestyle of a ruminal representative of the *Methanomassiliicoccales*. Stand. Genomic Sci. *11*, 59. https://doi.org/10.1186/s40793-016-0183-5

Liu, D., Nishida, M., Takahashi, T., and Asakawa, S. (2018). Transcription of *mcrA* gene decreases upon prolonged non-flooding period in a methanogenic archaeal community of a paddy-upland rotational field soil. Microb. Ecol. *75*, 751–760. https://doi.org/10.1007/s00248-017-1063-2

Liu, J., Xu, H., Jiang, Y., Zhang, K., Hu, Y., and Zeng, Z. (2017a). Methane emissions and microbial communities as influenced by dual cropping of *Azolla* along with early rice. Sci. Rep. *7*, 40635. https://doi.org/10.1038/srep40635

Liu, L., Xu, X., Cao, Y., Cai, C., Cui, H., and Yao, J. (2017b). Nitrate decreases methane production also

by increasing methane oxidation through stimulating NC10 population in ruminal culture. AMB Express *7*, 76. https://doi.org/10.1186/s13568-017-0377-2

Liu, Y., and Whitman, W.B. (2008). Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. Ann. N. Y. Acad. Sci. *1125*, 171–189. https://doi.org/10.1196/annals.1419.019

Lloyd, K. (2015). Microbiology. Beyond known methanogens. Science *350*, 384. https://doi.org/10.1126/science.aad4066

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

Loulergue, L., Schilt, A., Spahni, R., Masson-Delmotte, V., Blunier, T., Lemieux, B., Barnola, J.M., Raynaud, D., Stocker, T.F., and Chappellaz, J. (2008). Orbital and millennial-scale features of atmospheric CH4 over the past 800,000 years. Nature *453*, 383–386. https://doi.org/10.1038/nature06950

Lu, Y., and Conrad, R. (2005). In situ stable isotope probing of methanogenic archaea in the rice rhizosphere. Science *309*, 1088–1090.

Lü, Z., and Lu, Y. (2012). *Methanocella conradii* sp. nov., a thermophilic, obligate hydrogenotrophic methanogen, isolated from Chinese rice field soil. PLOS ONE *7*, e35279. https://doi.org/10.1371/journal.pone.0035279

Luesken, F.A., Wu, M.L., Op den Camp, H.J., Keltjens, J.T., Stunnenberg, H., Francoijs, K.J., Strous, M., and Jetten, M.S. (2012). Effect of oxygen on the anaerobic methanotroph '*Candidatus* Methylomirabilis oxyfera': kinetic and transcriptional analysis. Environ. Microbiol. *14*, 1024–1034. https://doi.org/10.1111/j.1462-2920.2011.02682.x

Lüke, C., and Frenzel, P. (2011). Potential of *pmoA* amplicon pyrosequencing for methanotroph diversity studies. Appl. Environ. Microbiol. *77*, 6305–6309. https://doi.org/10.1128/AEM.05355-11

Lyu, Z., and Lu, Y. (2018). Metabolic shift at the class level sheds light on adaptation of methanogens to oxidative environments. ISME J. *12*, 411–423. https://doi.org/10.1038/ismej.2017.173

Martinez-Cruz, K., Leewis, M.C., Herriott, I.C., Sepulveda-Jauregui, A., Anthony, K.W., Thalasso, F., and Leigh, M.B. (2017). Anaerobic oxidation of methane by aerobic methanotrophs in sub-Arctic lake sediments. Sci. Total Environ. 607-608, 23–31.

Maus, I., Rumming, M., Bergmann, I., Heeg, K., Pohl, M., Nettmann, E., Jaenicke, S., Blom, J., Pühler, A., Schlüter, A., *et al.* (2018). Characterization of *Bathyarchaeota* genomes assembled from metagenomes of biofilms residing in mesophilic and thermophilic biogas reactors. Biotechnol. Biofuels *11*, 167. https://doi.org/10.1186/s13068-018-1162-4

McCalley, C.K., Woodcroft, B.J., Hodgkins, S.B., Wehr, R.A., Kim, E.H., Mondav, R., Crill, P.M., Chanton, J.P., Rich, V.I., Tyson, G.W., *et al.* (2014). Methane dynamics regulated by microbial community response to permafrost thaw. Nature *514*, 478–481. https://doi.org/10.1038/nature13798

McGlynn, S.E. (2017). Energy metabolism during anaerobic methane oxidation in ANME archaea. Microbes Environ. *32*, 5–13. https://doi.org/10.1264/jsme2.ME16166

McGlynn, S.E., Chadwick, G.L., Kempes, C.P., and Orphan, V.J. (2015). Single cell activity reveals direct electron transfer in methanotrophic consortia. Nature *526*, 531–535. https://doi.org/10.1038/nature15512

McKay, L.J., Hatzenpichler, R., Inskeep, W.P., and Fields, M.W. (2017). Occurrence and expression of novel methyl-coenzyme M reductase gene (*mcrA*) variants in hot spring sediments. Sci. Rep. *7*, 7252. https://doi.org/10.1038/s41598-017-07354-x

Meng, H., Wang, Y.F., Chan, H.W., Wu, R.N., and Gu, J.D. (2016). Co-occurrence of nitrite-dependent anaerobic ammonium and methane oxidation processes in subtropical acidic forest soils. Appl. Microbiol. Biotechnol. *100*, 7727–7739. https://doi.org/10.1007/s00253-016-7585-6

Merkel, A.Y., Podosokorskaya, O.A., Sokolova, T.G., and Bonch-Osmolovskaya, E.A. (2016). Diversity of methanogenic archaea from the 2012 terrestrial hot spring (Valley of Geysers, Kamchatka). Microbiology *85*, 342–349. https://doi.org/10.1134/S0026261716030073

Milucka, J., Ferdelman, T.G., Polerecky, L., Franzke, D., Wegener, G., Schmid, M., Lieberwirth, I., Wagner, M., Widdel, F., and Kuypers, M.M. (2012). Zero-valent sulphur is a key intermediate in marine methane oxidation. Nature *491*, 541–546. https://doi.org/10.1038/nature11656

Milucka, J., Kirf, M., Lu, L., Krupke, A., Lam, P., Littmann, S., Kuypers, M.M., and Schubert, C.J. (2015). Methane oxidation coupled to oxygenic photosynthesis in anoxic waters. ISME J. *9*, 1991–2002. https://doi.org/10.1038/ismej.2015.12

Miyashita, A., Mochimaru, H., Kazama, H., Ohashi, A., Yamaguchi, T., Nunoura, T., Horikoshi, K., Takai, K., and Imachi, H. (2009). Development of 16S rRNA gene-targeted primers for detection of archaeal anaerobic methanotrophs (ANMEs). FEMS Microbiol. Lett. *297*, 31–37. https://doi.org/10.1111/j.1574-6968.2009.01648.x

Mohammadi, S., Pol, A., van Alen, T.A., Jetten, M.S., and Op den Camp, H.J. (2017). *Methylacidiphilum fumariolicum* SolV, a thermoacidophilic 'Knallgas' methanotroph with both an oxygen-sensitive and -insensitive hydrogenase. ISME J. *11*, 945–958. https://doi.org/10.1038/ismej.2016.171

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

Morris, S.A., Radajewski, S., Willison, T.W., and Murrell, J.C. (2002). Identification of the functionally active methanotroph population in a peat soil microcosm by stable-isotope probing. Appl. Environ. Microbiol. *68*, 1446–1453.

Murase, J., and Kimura, M. (1994). Methane production and its fate in paddy fields. VII. Electron acceptors responsible for anaerobic methane oxidation. Soil Sci. Plant Nutr. *40*, 647–654.

Murray, R.G., and Stackebrandt, E. (1995). Taxonomic note: implementation of the provisional status *Candidatus* for incompletely described procaryotes. Int. J. Syst. Bacteriol. *45*, 186–187. https://doi.org/10.1099/00207713-45-1-186

Myhre, G., Shindell, D., Bréon, F.-M., Collins, W., Fuglestveldt, J., Huang, J., Koch, D., Lamarque, J.-F., Lee, D., Mendoza, B., *et al.* (2013). Anthropogenic and natural radiative forcing. In Climate Change 2013: The Physical Science Basis Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, T.F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P.M. Midgley, eds. (Cambridge University Press, Cambridge, UK and New York, NY). https://doi.org/10.1017/CBO9781107415324.018

Naqvi, S.W.A., Lam, P., Narvenkar, G., Sarkar, A., Naik, H., Pratihary, A., Shenoy, D.M., Gauns, M., Kurian, S., Damare, S., *et al.* (2018). Methane stimulates massive nitrogen loss from freshwater reservoirs in India. Nat. Commun. *9*, 1265. https://doi.org/10.1038/s41467-018-03607-z

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

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

Niederberger, T.D., Perreault, N.N., Tille, S., Lollar, B.S., Lacrampe-Couloume, G., Andersen, D., Greer, C.W., Pollard, W., and Whyte, L.G. (2010). Microbial characterization of a subzero, hypersaline methane seep in the Canadian High Arctic. ISME J. *4*, 1326–1339. https://doi.org/10.1038/ismej.2010.57

Nobu, M.K., Narihiro, T., Kuroda, K., Mei, R., and Liu, W.T. (2016). Chasing the elusive Euryarchaeota class WSA2: genomes reveal a uniquely fastidious methyl-reducing methanogen. ISME J. *10*, 2478–2487. https://doi.org/10.1038/ismej.2016.33

Oren, A. (2014). The family *Methanosarcinaceae*. In The Prokaryotes, E. Rosenberg, E.F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, eds. (Springer, Berlin, Heidelberg), pp. 259–281. https://doi.org/10.1007/978-3-642-38954-2_408

Oswald, K., Milucka, J., Brand, A., Littmann, S., Wehrli, B., Kuypers, M.M., and Schubert, C.J. (2015). Light-dependent aerobic methane oxidation reduces methane emissions from seasonally stratified lakes. PLOS ONE *10*, e0132574. https://doi.org/10.1371/journal.pone.0132574

Oswald, K., Milucka, J., Brand, A., Hach, P., Littmann, S., Wehrli, B., Kuypers, M.M.M., and Schubert, C.J. (2016). Aerobic gammaproteobacterial methanotrophs mitigate methane emissions from oxic and anoxic lake waters. Limnol. Oceanogr. *61*, S101–S118. https://doi.org/10.1002/lno.10312

Oswald, K., Graf, J.S., Littmann, S., Tienken, D., Brand, A., Wehrli, B., Albertsen, M., Daims, H., Wagner, M., Kuypers, M.M., *et al.* (2017). *Crenothrix* are major methane consumers in stratified lakes. ISME J. *11*, 2124–2140. https://doi.org/10.1038/ismej.2017.77

Padilla, C.C., Bristow, L.A., Sarode, N., Garcia-Robledo, E., Gómez Ramírez, E., Benson, C.R., Bourbonnais, A., Altabet, M.A., Girguis, P.R., Thamdrup, B., *et al.* (2016). NC10 bacteria in marine oxygen minimum zones. ISME J. *10*, 2067–2071. https://doi.org/10.1038/ismej.2015.262

Pandit, P.S., Hoppert, M., and Rahalkar, M.C. (2018). Description of *Methylocucumis oryzae* gen. nov., sp. nov., a type I methanotroph with large cells and pale pink colour, isolated from an Indian rice field. Anton. Leeuw. *111*, 2473–2484. https://doi.org/10.1007/s10482-018-1136-3

Parks, D.H., Rinke, C., Chuvochina, M., Chaumeil, P.A., Woodcroft, B.J., Evans, P.N., Hugenholtz, P., and Tyson, G.W. (2017). Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. Nat. Microbiol. *2*, 1533–1542. https://doi.org/10.1038/s41564-017-0012-7

Paul, K., Nonoh, J.O., Mikulski, L., and 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. https://doi.org/10.1128/AEM.02193-12

Peters, V., and Conrad, R. (1995). Methanogenic and other strictly anaerobic bacteria in desert soil and other oxic soils. Appl. Environ. Microbiol. *61*, 1673–1676.

Praeg, N., Wagner, A.O., and Illmer, P. (2014). Effects of fertilisation, temperature and water content on microbial properties and methane production and methane oxidation in subalpine soils. Eur. J. Soil Biol. *65*, 96–106. https://doi.org/10.1016/j.ejsobi.2014.10.002

Pratscher, J., Dumont, M.G., and Conrad, R. (2011). Assimilation of acetate by the putative atmospheric methane oxidizers belonging to the USC alpha clade. Environ. Microbiol. *13*, 2692–2701. https://doi.org/10.1111/j.1462-2920.2011.02537.x

Pratscher, J., Vollmers, J., Wiegand, S., Dumont, M.G., and Kaster, A.K. (2018). Unravelling the identity, metabolic potential and global biogeography of the atmospheric methane-oxidizing upland soil cluster α. Environ. Microbiol. *20*, 1016–1029. https://doi.org/10.1111/1462-2920.14036

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

Raghoebarsing, A.A., Pol, A., van de Pas-Schoonen, K.T., Smolders, A.J., Ettwig, K.F., Rijpstra, W.I., Schouten, S., Damsté, J.S., Op den Camp, H.J., Jetten, M.S., *et al.* (2006). A microbial consortium couples anaerobic methane oxidation to denitrification. Nature *440*, 918–921.

Rahalkar, M., Bussmann, I., and Schink, B. (2007). *Methylosoma difficile* gen. nov., sp nov., a novel methanotroph enriched by gradient cultivation from littoral sediment of Lake Constance. Int. J. Syst. Evol. Microbiol. *57*, 1073–1080. https://doi.org/10.1099/ijs.0.64574-0

Rahalkar, M.C., Pandit, P.S., Dhakephalkar, P.K., Pore, S., Arora, P., and Kapse, N. (2016). Genome characteristics of a novel type I methanotroph (Sn10-6) isolated from a flooded Indian rice field. Microb. Ecol. *71*, 519–523. https://doi.org/10.1007/s00248-015-0699-z

Reeburgh, W.S. (2003). Global methane biogeochemistry. In Treatise on Geochemistry: The Atmosphere, R.F. Keeling, H.D. Holland, and K.K. Turekian, eds. (Elsevier-Pergamon, Oxford, UK), pp. 65–89. https://doi.org/10.1016/B0-08-043751-6/04036-6

Reed, D.C., Deemer, B.R., van Grinsven, S., and Harrison, J.A. (2017). Are elusive anaerobic pathways key methane sinks in eutrophic lakes and reservoirs? Biogeochem. *134*, 29–39. https://doi.org/10.1007/s10533-017-0356-3

Reim, A., Lüke, C., Krause, S., Pratscher, J., and Frenzel, P. (2012). One millimetre makes the difference: high-resolution analysis of methane-oxidizing bacteria and their specific activity at the oxic-anoxic interface in a flooded paddy soil. ISME J. *6*, 2128–2139. https://doi.org/10.1038/ismej.2012.57

Reim, A., Hernández, M., Klose, M., Chidthaisong, A., Yuttitham, M., and Conrad, R. (2017). Response of methanogenic microbial communities to desiccation stress in flooded and rain-fed paddy soil from Thailand. Front. Microbiol. *8*, 785. https://doi.org/10.3389/fmicb.2017.00785

Ricke, P., Kube, M., Nakagawa, S., Erkel, C., Reinhardt, R., and Liesack, W. (2005). First genome data from uncultured upland soil cluster alpha methanotrophs provide further evidence for a close phylogenetic relationship to *Methylocapsa acidiphila* B2 and for high-affinity methanotrophy involving particulate methane monooxygenase. Appl. Environ. Microbiol. *71*, 7472–7482.

Rissanen, A.J., Karvinen, A., Nykänen, H., Peura, S., Tiirola, M., Mäki, A., and Kankaala, P. (2017). Effects of alternative electron acceptors on the activity and community structure of methane-producing and consuming microbes in the sediments of two shallow boreal lakes. FEMS Microbiol. Ecol. *93*. https://doi.org/10.1093/femsec/fix078

Rissanen, A.J., Saarenheimo, J., Tiirola, M., Peura, S., Aalto, S.L., Karvinen, A., and Nykanen, H. (2018). Gammaproteobacterial methanotrophs dominate methanotrophy in aerobic and anaerobic layers of boreal lake waters. Aquat. Microb. Ecol. *81*, 257–276. https://doi.org/10.3354/ame01874

Roslev, P., and King, G.M. (1994). Survival and recovery of methanotrophic bacteria starved under oxic and anoxic conditions. Appl. Environ. Microbiol. *60*, 2602–2608.

Saunois, M., Bousquet, P., Poulter, B., Peregon, A., Ciais, P., Canadell, J.G., Dlugokencky, E.J., Etiope, G., Bastviken, D., Houweling, S., *et al.* (2016a). The global methane budget 2000–2012. Earth Syst. Sci. Data *8*, 697–751. https://doi.org/10.5194/essd-8-697-2016

Saunois, M., Jackson, R.B., Bousquet, P., Poulter, B., and Canadell, J.G. (2016b). The growing role of methane in anthropogenic climate change. Environ. Res. Lett. *11*, 120207. https://doi.org/10.1088/1748-9326/11/12/120207

Scheller, S., Goenrich, M., Boecher, R., Thauer, R.K., and Jaun, B. (2010). The key nickel enzyme of methanogenesis catalyses the anaerobic oxidation of methane. Nature *465*, 606–608. https://doi.org/10.1038/nature09015

Scheller, S., Yu, H., Chadwick, G.L., McGlynn, S.E., and Orphan, V.J. (2016). Artificial electron acceptors decouple archaeal methane oxidation from sulfate reduction. Science *351*, 703–707. https://doi.org/10.1126/science.aad7154

Schubert, C.J., Coolen, M.J., Neretin, L.N., Schippers, A., Abbas, B., Durisch-Kaiser, E., Wehrli, B., Hopmans, E.C., Damsté, J.S., Wakeham, S., *et al.* (2006). Aerobic and anaerobic methanotrophs in the Black Sea water column. Environ. Microbiol. *8*, 1844–1856.

Scott, D.T., McKnight, D.M., Blunt-Harris, E.L., Kolesar, S.E., and Lovley, D.R. (1998). Quinone moieties act as electron acceptors in the reduction of humic substances by humics-reducing microorganisms. Environ. Sci. Technol. *32*, 2984–2989. https://doi.org/10.1021/es980272q

Segarra, K.E., Schubotz, F., Samarkin, V., Yoshinaga, M.Y., Hinrichs, K.U., and Joye, S.B. (2015). High rates of anaerobic methane oxidation in freshwater wetlands reduce potential atmospheric methane emissions. Nat. Commun. *6*, 7477. https://doi.org/10.1038/ncomms8477

Semrau, J.D., DiSpirito, A.A., and Yoon, S. (2010). Methanotrophs and copper. FEMS Microbiol. Rev. *34*, 496–531. https://doi.org/10.1111/j.1574-6976.2010.00212.x

Serrano-Silva, N., Sarria-Guzmán, Y., Dendooven, L., and Luna-Guido, M. (2014). Methanogenesis and methanotrophy in soil: a review. Pedosphere *24*, 291–307. https://doi.org/10.1016/S1002-0160(14)60016-3

Shen, L.D., Liu, S., Huang, Q., Lian, X., He, Z.F., Geng, S., Jin, R.C., He, Y.F., Lou, L.P., Xu, X.Y., *et al.* (2014). Evidence for the cooccurrence of nitrite-dependent anaerobic ammonium and methane oxidation processes in a flooded paddy field. Appl. Environ. Microbiol. *80*, 7611–7619. https://doi.org/10.1128/AEM.02379-14

Shen, L.D., He, Z.F., Wu, H.S., and Gao, Z.Q. (2015a). Nitrite-dependent anaerobic methane-oxidising bacteria: unique microorganisms with special properties. Curr. Microbiol. *70*, 562–570. https://doi.org/10.1007/s00284-014-0762-x

Shen, L.D., Huang, Q., He, Z.F., Lian, X., Liu, S., He, Y.F., Lou, L.P., Xu, X.Y., Zheng, P., and Hu, B.L. (2015b). Vertical distribution of nitrite-dependent anaerobic methane-oxidising bacteria in natural freshwater wetland soils. Appl. Microbiol. Biotechnol. *99*, 349–357. https://doi.org/10.1007/s00253-014-6031-x

Shen, L.D., Liu, S., He, Z.F., Lian, X., Huang, Q., He, Y.F., Lou, L.P., Xu, X.Y., Zheng, P., and Hu, B.L. (2015c). Depth-specific distribution and importance of nitrite-dependent anaerobic ammonium and methane-oxidising bacteria in an urban wetland. Soil Biol. Biochem. *83*, 43–51. https://doi.org/10.1016/j.soilbio.2015.01.010

Shen, L.D., Wu, H.S., and Gao, Z.Q. (2015d). Distribution and environmental significance of nitrite-dependent anaerobic methane-oxidising bacteria in natural ecosystems. Appl. Microbiol. Biotechnol. *99*, 133–142. https://doi.org/10.1007/s00253-014-6200-y

Shen, L.D., Wu, H.S., Gao, Z.Q., Li, J., and Liu, X. (2016a). Presence of diverse *Candidatus* Methylomirabilis oxyfera-like bacteria of NC10 phylum in agricultural soils. J. Appl. Microbiol. *120*, 1552–1560. https://doi.org/10.1111/jam.13119

Shen, L.D., Wu, H.S., Gao, Z.Q., Liu, X., and Li, J. (2016b). Comparison of community structures of *Candidatus* Methylomirabilis oxyfera-like bacteria of NC10 phylum in different freshwater habitats. Sci. Rep. *6*, 25647. https://doi.org/10.1038/srep25647

Shiau, Y.-J., Cai, Y., Jia, Z., Chen, C.-L., and Chiu, C.-Y. (2018). Phylogenetically distinct methanotrophs modulate methane oxidation in rice paddies across Taiwan. Soil Biol. Biochem. *124*, 59–69. https://doi.org/10.1016/j.soilbio.2018.05.025

Singleton, C.M., McCalley, C.K., Woodcroft, B.J., Boyd, J.A., Evans, P.N., Hodgkins, S.B., Chanton, J.P., Frolking, S., Crill, P.M., Saleska, S.R., *et al.* (2018). Methanotrophy across a natural permafrost thaw environment. ISME J. *12*, 2544–2558. https://doi.org/10.1038/s41396-018-0065-5

Skennerton, C.T., Ward, L.M., Michel, A., Metcalfe, K., Valiente, C., Mullin, S., Chan, K.Y., Gradinaru, V., and Orphan, V.J. (2015). Genomic reconstruction of an uncultured hydrothermal vent gammaproteobacterial methanotroph (family *Methylothermaceae*) indicates multiple adaptations to oxygen limitation. Front. Microbiol. *6*, 1425. https://doi.org/10.3389/fmicb.2015.01425

Smemo, K.A., and Yavitt, J.B. (2007). Evidence for anaerobic CH4 oxidation in freshwater peatlands. Geomicrobiology *24*, 583–597. https://doi.org/10.1080/01490450701672083

Smemo, K.A., and Yavitt, J.B. (2011). Anaerobic oxidation of methane: an underappreciated aspect of methane cycling in peatland ecosystems? Biogeoscience *8*, 779–793. https://doi.org/10.5194/bg-8-779-2011

Söllinger, A., Schwab, C., Weinmaier, T., Loy, A., Tveit, A.T., Schleper, C., and Urich, T. (2016). Phylogenetic and genomic analysis of *Methanomassiliicoccales* in wetlands and animal intestinal tracts reveals clade-specific habitat preferences. FEMS Microbiol. Ecol. *92*, fiv149. https://doi.org/10.1093/femsec/fiv149

Sorokin, D.Y., Makarova, K.S., Abbas, B., Ferrer, M., Golyshin, P.N., Galinski, E.A., Ciordia, S., Mena, M.C., Merkel, A.Y., Wolf, Y.I., *et al.* (2017). Discovery of extremely halophilic, methyl-reducing euryarchaea provides insights into the evolutionary origin of methanogenesis. Nat. Microbiol. *2*, 17081. https://doi.org/10.1038/nmicrobiol.2017.81

Sorokin, D.Y., Merkel, A.Y., Abbas, B., Makarova, K.S., Rijpstra, W.I.C., Koenen, M., Sinninghe Damsté, J.S., Galinski, E.A., Koonin, E.V., and van Loosdrecht, M.C.M. (2018). *Methanonatronarchaeum thermophilum* gen. nov., sp. nov. and *'Candidatus* Methanohalarchaeum thermophilum', extremely halo(natrono)philic methyl-reducing methanogens from hypersaline lakes comprising a new euryarchaeal class *Methanonatronarchaeia* classis nov. Int. J. Syst. Evol. Microbiol. *68*, 2199–2208. https://doi.org/10.1099/ijsem.0.002810

Stoecker, K., Bendinger, B., Schöning, B., Nielsen, P.H., Nielsen, J.L., Baranyi, C., Toenshoff, E.R., Daims, H., and Wagner, M. (2006). Cohn's Crenothrix is a filamentous methane oxidizer with an unusual methane monooxygenase. Proc. Natl. Acad. Sci. U.S.A. *103*, 2363–2367. https://doi.org/10.1073/pnas.0506361103

Takeuchi, M., Yoshioka, H., Seo, Y., Tanabe, S., Tamaki, H., Kamagata, Y., Takahashi, H.A., Igari, S., Mayumi, D., and Sakata, S. (2011). A distinct freshwater-adapted subgroup of ANME-1 dominates active archaeal communities in terrestrial subsurfaces in Japan. Environ. Microbiol. *13*, 3206–3218. https://doi.org/10.1111/j.1462-2920.2011.02517.x

Takeuchi, M., Katayama, T., Yamagishi, T., Hanada, S., Tamaki, H., Kamagata, Y., Oshima, K., Hattori, M., Marumo, K., Nedachi, M., *et al.* (2014). *Methyloceanibacter caenitepidi* gen. nov., sp nov., a facultatively methylotrophic bacterium isolated from marine sediments near a hydrothermal vent. Int. J. Syst. Evol. Microbiol. *64*, 462–468. https://doi.org/10.1099/ijs.0.053397-0

Tang, K.W., McGinnis, D.F., Frindte, K., Bruchert, V., and Grossart, H.P. (2014). Paradox reconsidered: Methane oversaturation in well-oxygenated lake waters. Limnol. Oceanogr. *59*, 275–284. https://doi.org/10.4319/lo.2014.59.1.0275

Teh, Y.A., Silver, W.L., and Conrad, M.E. (2005). Oxygen effects on methane production and oxidation in humid tropical forest soils. Global Change Biol. *11*, 1283–1297. https://doi.org/10.1111/j.1365-2486.2005.00983.x

Thauer, R.K., and Shima, S. (2008). Methane as fuel for anaerobic microorganisms. Ann. N. Y. Acad. Sci. *1125*, 158–170.

Thauer, R.K., Kaster, A.K., Seedorf, H., Buckel, W., and Hedderich, R. (2008). Methanogenic archaea: ecologically relevant differences in energy conservation. Nat. Rev. Microbiol. *6*, 579–591. https://doi.org/10.1038/nrmicro1931

Tholen, A., Pester, M., and Brune, A. (2007). Simultaneous methanogenesis and oxygen reduction by *Methanobrevibacter cuticularis* at low oxygen fluxes. FEMS Microbiol. Ecol. *62*, 303–312.

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

Timmers, P.H., Welte, C.U., Koehorst, J.J., Plugge, C.M., Jetten, M.S., and Stams, A.J. (2017). Reverse methanogenesis and respiration in methanotrophic Archaea. Archaea *2017*, 1654237. https://doi.org/10.1155/2017/1654237

Tveit, A., Schwacke, R., Svenning, M.M., and Urich, T. (2013). Organic carbon transformations in high-Arctic peat soils: key functions and microorganisms. ISME J. *7*, 299–311. https://doi.org/10.1038/ismej.2012.99

Tveit, A.T., Urich, T., and Svenning, M.M. (2014). Metatranscriptomic analysis of arctic peat soil microbiota. Appl. Environ. Microbiol. *80*, 5761–5772. https://doi.org/10.1128/AEM.01030-14

Vaksmaa, A., Lüke, C., van Alen, T., Valè, G., Lupotto, E., Jetten, M.S., and Ettwig, K.F. (2016). Distribution and activity of the anaerobic methanotrophic community in a nitrogen-fertilized Italian paddy soil. FEMS Microbiol. Ecol. *92*, fiw181.

Vaksmaa, A., Guerrero-Cruz, S., van Alen, T.A., Cremers, G., Ettwig, K.F., Lüke, C., and Jetten, M.S.M. (2017a). Enrichment of anaerobic nitrate-dependent methanotrophic '*Candidatus* Methanoperedens nitroreducens' archaea from an Italian paddy field soil. Appl. Microbiol. Biotechnol. *101*, 7075–7084. https://doi.org/10.1007/s00253-017-8416-0

Vaksmaa, A., Jetten, M.S., Ettwig, K.F., and Lüke, C. (2017b). *McrA* primers for the detection and quantification of the anaerobic archaeal methanotroph '*Candidatus* Methanoperedens nitroreducens'. Appl. Microbiol. Biotechnol. *101*, 1631–1641. https://doi.org/10.1007/s00253-016-8065-8

Vaksmaa, A., van Alen, T.A., Ettwig, K.F., Lupotto, E., Valè, G., Jetten, M.S.M., and Lüke, C. (2017c). Stratification of diversity and activity of methanogenic and methanotrophic microorganisms in a nitrogen-fertilized Italian paddy soil. Front. Microbiology *8*, 2127. https://doi.org/10.3389/fmicb.2017.02127

van Kessel, M.A., Speth, D.R., Albertsen, M., Nielsen, P.H., Op den Camp, H.J., Kartal, B., Jetten, M.S., and Lücker, S. (2015). Complete nitrification by a single microorganism. Nature *528*, 555–559. https://doi.org/10.1038/nature16459

van Kessel, M.A., Stultiens, K., Slegers, M.F., Guerrero Cruz, S., Jetten, M.S., Kartal, B., and Op den Camp, H.J. (2018). Current perspectives on the application of N-damo and anammox in wastewater treatment. Curr. Opin. Biotechnol. *50*, 222–227.

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

Vekeman, B., Kerckhof, F.M., Cremers, G., de Vos, P., Vandamme, P., Boon, N., Op den Camp, H.J., and Heylen, K. (2016). New *Methyloceanibacter* diversity from North Sea sediments includes methanotroph containing solely the soluble methane monooxygenase. Environ. Microbiol. *18*, 4523–4536. https://doi.org/10.1111/1462-2920.13485

Vinogradov, S.N., Tinajero-Trejo, M., Poole, R.K., and Hoogewijs, D. (2013). Bacterial and archaeal globins - a revised perspective. Biochim. Biophys. Acta *1834*, 1789–1800. https://doi.org/10.1016/j.bbapap.2013.03.021

Wang, B., Lerdau, M., and He, Y. (2017). Widespread production of nonmicrobial greenhouse gases in soils. Glob. Chang. Biol. *23*, 4472–4482. https://doi.org/10.1111/gcb.13753

Wang, Z.P., Chang, S.X., Chen, H., and Han, X.G. (2013). Widespread non-microbial methane production by organic compounds and the impact of environmental stresses. Earth Sci. Rev. *127*, 193–202. https://doi.org/10.1016/j.earscirev.2013.10.001

Wegener, G., Krukenberg, V., Riedel, D., Tegetmeyer, H.E., and Boetius, A. (2015). Intercellular wiring enables electron transfer between methanotrophic archaea and bacteria. Nature *526*, 587–590. https://doi.org/10.1038/nature15733

Welte, C.U. (2018). Revival of archaeal methane microbiology. mSystems *3*, e00181–17. https://doi.org/10.1128/mSystems.00181-17

Welte, C.U., Rasigraf, O., Vaksmaa, A., Versantvoort, W., Arshad, A., Op den Camp, H.J., Jetten, M.S., Lüke, C., and Reimann, J. (2016). Nitrate- and nitrite-dependent anaerobic oxidation of methane. Environ. Microbiol. Rep. *8*, 941–955. https://doi.org/10.1111/1758-2229.12487

Whitman, W.B., Bowden, T.L., and Boone, D.R. (2014). The methanogenic bacteria. In The Prokaryotes, E. Rosenberg, E.F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, eds. (Springer, Berlin, Heidelberg), pp. 123–163. https://doi.org/10.1007/0-387-30743-5_9

Whitman, W.B., Woyke, T., Klenk, H.P., Zhou, Y., Lilburn, T.G., Beck, B.J., De Vos, P., Vandamme, P., Eisen, J.A., Garrity, G., *et al.* (2015). Genomic encyclopedia of bacterial and archaeal type strains, phase III: the genomes of soil and plant-associated and newly described type strains. Stand. Genomic Sci. *10*, 26. https://doi.org/10.1186/s40793-015-0017-x

Winkel, M., Mitzscherling, J., Overduin, P.P., Horn, F., Winterfeld, M., Rijkers, R., Grigoriev, M.N., Knoblauch, C., Mangelsdorf, K., Wagner, D., *et al.* (2018). Anaerobic methanotrophic communities thrive in deep submarine permafrost. Sci. Rep. *8*, 1291. https://doi.org/10.1038/s41598-018-19505-9

Wu, M.L., de Vries, S., van Alen, T.A., Butler, M.K., Op den Camp, H.J., Keltjens, J.T., Jetten, M.S., and Strous, M. (2011). Physiological role of the respiratory quinol oxidase in the anaerobic nitrite-reducing methanotroph '*Candidatus* Methylomirabilis oxyfera'. Microbiology *157*, 890–898. https://doi.org/10.1099/mic.0.045187-0

Xie, W., Jiao, N., Ma, C., Fang, S., Phelps, T.J., Zhu, R., and Zhang, C. (2017). The response of archaeal species to seasonal variables in a subtropical aerated soil: insight into the low abundant methanogens. Appl. Microbiol. Biotechnol. *101*, 6505–6515. https://doi.org/10.1007/s00253-017-8349-7

Xu, S., Cai, C., Guo, J., Lu, W., Yuan, Z., and Hu, S. (2018). Different clusters of *Candidatus* 'Methanoperedens nitroreducens'-like archaea as revealed by high-throughput sequencing with new primers. Sci. Rep. *8*, 7695. https://doi.org/10.1038/s41598-018-24974-z

Yuan, Y., Conrad, R., and Lu, Y. (2011). Transcriptional response of methanogen *mcrA* genes to oxygen exposure of rice field soil. Environ. Microbiol. Rep. *3*, 320–328. https://doi.org/10.1111/j.1758-2229.2010.00228.x

Zhu, B., van Dijk, G., Fritz, C., Smolders, A.J., Pol, A., Jetten, M.S., and Ettwig, K.F. (2012). Anaerobic oxidization of methane in a minerotrophic peatland: enrichment of nitrite-dependent methane-oxidizing bacteria. Appl. Environ. Microbiol. *78*, 8657–8665. https://doi.org/10.1128/AEM.02102-12

Zhu, G., Zhou, L., Wang, Y., Wang, S., Guo, J., Long, X.E., Sun, X., Jiang, B., Hou, Q., Jetten, M.S., *et al.* (2015). Biogeographical distribution of denitrifying anaerobic methane oxidizing bacteria in Chinese wetland ecosystems. Environ. Microbiol. Rep. *7*, 128–138. https://doi.org/10.1111/1758-2229.1221