PEDOSPHERE（土壤圈英文版）

Pedosphere ISSN 1002-0160/CN 32-1315/P doi:10.1016/S1002-0160(18)60052-9

Importance of hydrogenotrophic, aceticlastic, and methylotrophic methanogenesis for CH_4 production in terrestrial aquatic environments

Ralf Conrad

Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Str.10, 35043 Marburg, (Germany)

ABSTRACT

Microbial methanogenesis is the major source of the greenhouse gas methane. It is the final step in the anaerobic degradation of organic matter when inorganic electron acceptors such as nitrate, ferric iron or sulfate have been depleted. Knowledge of the degradation pathway is important for creation of mechanistic models, prediction of future CH_4 emission scenarios and development of mitigation strategies. In most terrestrial environments CH_4 is produced from either acetate (aceticlastic methanogenesis) or H_2 plus CO_2 (hydrogenotrophic methanogenesis). Hydrogen can be replaced by other CO_2-type methanogenesis, using formate, CO or alcohols as substrates. The ratio of these two pathways is tightly constrained by the stoichiometry of conversion processes, if the degradation of organic matter is complete (e.g. degradation of straw in rice paddies). Then, fermentation eventually results in production of acetate and H_2 at a ratio, which allows >67% aceticlastic and <33% hydrogenotrophic methanogensis. However, acetate production can be favored when heterotrophic or chemolithotrophic acetogenesis is enhanced, and H_2 production can be favored when syntrophic acetate oxidation is enhanced. This typically happens at low and elevated temperatures, respectively. Thus, temperature can strongly influence the methanogenic pathway, which may range from 100% aceticlastic methanogenesis at low temperatures to 100% hydrogenotrophic methanogenesis at high temperatures. However, if the degradation of organic matter is not complete (e.g., degradation of soil organic matter), the stoichiometry of fermentation is not tightly constrained, resulting for example in the preferential production of H_2 followed by hydrogenotrophic methanogenesis. Preferential production of CH_4 by either aceticlastic or hydrogenotrophic methanogenesis can also happen, if one of the methanogenic substrates is not consumed by the methanogens but is instead accumulating, is volatilized or is otherwise utilized. Methylotrophic methanogens, which can use methanol as substrate, are widespread, but it is unlikely that methanol is produced in similar quantities as acetate, CO_2 and H_2. Methylotrophic methanogenesis is important in saline environments, where compatible solutes are degraded to methyl compounds (trimethyl amine, dimethyl sulfide), which then serve as non-competitive substrates, while acetate and hydrogen are degraded by non-methanogenic processes, e.g., sulfate reduction.

INTRODUCTION

Methanogenesis is the final step in the anaerobic microbial degradation of organic matter yielding methane and carbon dioxide as end products. Anaerobic microbial methane production (AMMP) is the only processes that allows complete mineralization of organic matter in the absence of inorganic oxidants such as O_2, nitrate, sulfate, ferric iron etc. In the Precambrium it was the almost exclusive process recycling dead biomass (Holland et al., 1986), and even in

our contemporary oxygenated biosphere it still accounts for about 1-2% of the total carbon cycle (Ehhalt, 1979). In the terrestrial biosphere AMMP nowadays occurs in aquatic environments, such as wetlands, flooded rice fields, and lake sediments. In addition it operates in the guts of many (but not all) animals (Hackstein et al., 1996) and in man-made systems like anaerobic digestors and landfills. These environments and process sites account for more than 60% of the global methane budget, which amounts to about 500-600 Tg a^{-1} (Conrad, 2009). In principle, AMMP may occur wherever degradable organic matter exists under anoxic (no O_2) conditions.

Operation of AMMP requires a complex community of different microorganisms consisting of various taxonomic groups that fulfill the following functions (Zehnder, 1978; Zinder, 1993; Schink and Stams, 2013; Conrad, 1999): (1) hydrolysis of polymeric organic matter (e.g., polysaccharide, protein, lipid) to monomers, followed by fermentation of the monomers to simple compounds, usually short-chain fatty acids (including acetate and formate), alcohols, H_2 and CO_2; (2) methanogenic conversion of acetate to CH_4 and CO_2; (3) methanogenic conversion of H_2 and CO_2 to CH_4; (4) syntrophic conversion of short-chain fatty acids (including acetate) and alcohols to acetate, CO_2 and H_2; (5) fermentation of monomers to only acetate (heterotrophic acetogenesis) or conversion of H_2 and CO_2 to acetate (chemolithotrophic acetogenesis) (Fig. 1A). The functions 1, 4 and 5 are usually accomplished by Bacteria, while functions 2 and 3 are accomplished by Archaea. Functions 2 and 3 are termed aceticlastic and hydrogenotrophic methanogenesis, respectively. The ratio at which these two methanogenic functions contribute to total CH_4 production depends on the relative net production rates of the two methane precursors acetate and H_2. Carbon dioxide, which is also required for hydrogenotrophic methanogenesis, is usually not a limiting substrate in terrestrial aquatic environments. The relative turnover rates of acetate and H_2 and the resulting ratio of aceticlastic and hydrogenotrophic methanogenesis are complex and depend on many different factors in the environment; they are subject of the present minireview. In addition, the role of methylotrophic methanogenesis will be discussed.

Fig. 1

Fig. 1 Schemes of anaerobic microbial methane production (AMMP) from organic matter (e.g., cellulose) by involving the following functions (numbers in squares): (1) hydrolysis of polymeric organic matter to monomers, followed by fermentation of the monomers to short-chain fatty acids, H_2 and CO_2; (2) methanogenic conversion of acetate to CH_4 and CO_2; (3) methanogenic conversion of H_2 and CO_2 to CH_4; (4) syntrophic conversion of short-chain fatty acids to acetate, CO_2 and H_2, or of acetate to CO_2 and H_2; (5) fermentation of monomers to only acetate (heterotrophic acetogenesis) or conversion of H_2 and CO_2 to acetate (chemolithotrophic acetogenesis). (A) Complete degradation with only negligible acetogenesis (function 5); (B) Compete degradation with acetogenesis (function 5) as main fermentation process; (C) Complete degradation with aceticlastic methanogenesis (function 2) being largely replaced by syntrophic acetate oxidation (function 4); (D) incomplete degradation of organic matter; (E) Complete degradation with accumulation of acetate; (F) Complete degradation with hydrogenotrophic methanogenesis (function 3) being outcompeted by iron reduction, sulfate reduction, etc.

Why is it important to know the relative contribution of hydrogenotrophic and aceticlastic methanogenesis, i.e. the methanogenic pathway? Besides pure scholastic curiosity, it is the necessity to understand all the details of the complex methane production pathway to be able to create mechanistic models. Such models are required for predictions of future CH_4 emission rates, including the emission of CH_4 carbon isotopes (Bridgham et al., 2013; VanBodegom and Scholten, 2001; Walter and Heimann, 2000; Vavilin, 2012; Xu et al., 2015). Atmospheric

isotope budgets are helpful constraints for quantifying the global CH_4 cycle and the carbon cycle in general (Brownlow et al., 2017; Tyler et al., 2007). Knowledge of process details is also required for the development of suitable mitigation strategies, since individual reactions may serve as targets to reduce CH_4 emission without compromising other important ecosystem functions. A good example is the successful reduction of CH_4 production in rumen fermentation without compromising animal health (Duin et al., 2016; Hristov et al., 2015).

Theoretical constraints

The complete anaerobic degradation of organic matter to CH_4 plus CO_2 is constrained by the nature of the organic material and by stoichiometry. Polysaccharides (e.g., cellulose, xylan) are arguably the most important class of dead plant biomass. Polysaccharides are relatively easily degraded by AMMP. For example, 80-90% of the rice straw that is added to flooded paddy soils is degraded within the first growth season (Neue and Scharpenseel, 1987).

If we assume complete degradation by AMMP, e.g., of cellulose, we arrive at the following stoichiometric equations:

$C_6H_{12}O_6 + 2\ H_2O$	→	$2\ CH_3COOH + 2\ CO_2 + 4\ H_2$	(1)
$2\ CH_3COOH$	→	$2\ CH_4 + 2\ CO_2$	(2)
$4\ H_2 + CO_2$	→	$CH_4 + 2\ H_2O$	(3)
$C_6H_{12}O_6$	→	$3\ CH_4 + 3\ CO_2$	(4)

Note that equation (1) encompasses all possible hydrolysis and fermentation reactions (function 1) and also all possible syntrophic conversions (function 4). However, it assumes that acetogenesis (function 5) is negligible (Fig. 1A). From the stoichiometric equations (1-4) one can conclude that complete degradation of cellulose by AMMP results in equal amounts of CH_4 and CO_2, and that of the 3 CH_4 produced in total, two are derived from aceticlastic methanogenesis (67%) and one is derived from hydrogenotrophic methanogenesis (33%).

Now let us assume that fermentation of cellulose occurs mainly by acetogenesis (function 5), the other fermentation processes (functions 1 and 4) being negligible:

$C_6H_{12}O_6$	→	$3\ CH_3COOH$	(5)
$3\ CH_3COOH$	→	$3\ CH_4 + 3\ CO_2$	(2)
$C_6H_{12}O_6$	→	$3\ CH_4 + 3\ CO_2$	(4)

We notice that under these conditions CH_4 is almost exclusively (100%) produced by aceticlastic methanogenesis (Fig. 1B).

It is also possible that AMMP is almost exclusively hydrogenotrophic, if organic matter is degraded to H_2 plus CO_2, and no acetate is produced. However, such degradation is only weakly exergonic under standard conditions. Conversion of glucose to 6 CO_2 and 12 H_2 only gives a $\Delta G^{o'} = -25$ kJ. Such process is thermodynamically equivalent to the operation of syntrophic acetate oxidation (equation 6) converting the initially produced acetate to H_2 plus CO_2 (Fig. 1C):

$C_6H_{12}O_6 + 2\ H_2O$	→	$2\ CH_3COOH + 2\ CO_2 + 4\ H_2$	(1)
$2\ CH_3COOH + 4\ H_2O$	→	$4\ CO_2 + 8\ H_2$	(6)
$12\ H_2 + CO_2$	→	$3\ CH_4 + 6\ H_2O$	(3)
$C_6H_{12}O_6$	→	$3\ CH_4 + 3\ CO_2$	(4)

Hence, complete cellulose degradation by AMMP may occur via all combinations of hydrogenotrophic plus aceticlastic methanogenesis.

Since syntrophic acetate oxidation is probably comparatively rare in nature (but see below), degradation of cellulose and of organic matter in general seems to predominantly occur by

aceticlastic methanogenesis. Indeed, early studies on anaerobic digestion have found AMMP operating at a ratio of about 67% aceticlastic and 33% hydrogenotrophic methanogenesis (Smith and Mah, 1966). Such ratio has also been found during early studies of anoxic rice field soil (Takai, 1970) and lake sediments (Cappenberg and Prins, 1974; Winfrey and Zeikus, 1979). Therefore, such pattern of AMMP has been considered the norm (Fig. 1A). However, in a former review (Conrad 1999) I have pointed out that exceptions of the general pattern of AMMP do exist. Since the last 20 years many more studies have been done, in particular including analysis of stable isotopes and molecular analyses of microbial communities, so that a new minireview of the regulation of the methanogenic pathways during AMMP is warranted.

Regulation by temperature

The relative contribution of hydrogenotrophic methanogenesis has been found to decrease with decreasing temperature, both in lake sediment (Glissmann et al., 2004; Schulz and Conrad, 1996) and rice field soil (Fey and Conrad, 2000). This effect is expressed in the physiological range of about 4 to 40°C. Thus, it has been found that H_2-consuming chemolithotrophic acetogenic bacteria outcompete methanogens at low temperature (Nozhevnikova et al., 1994; Conrad et al., 1989; Fu et al., 2018). Hence, acetate production is stimulated at low temperature in methanogenic environments such as rice field soil or lake sediments, while hydrogenotrophic methanogens are suppressed (Nozhevnikova et al., 2007). This happens despite the fact that hydrogenotrophic methanogenesis is thermodynamically more favorable than chemolithotrophic acetogenesis (Conrad and Wetter, 1990; Kotsyurbenko et al., 2001). The reason why acetogenic bacteria seem to be more psychrotolerant than methanogenic archaea is unknown, but one might speculate that it is caused by the different membrane structures of Bacteria (ester lipids) versus Archaea (ether lipids) (Valentine, 2007). At low temperature ester lipids can be more flexible than ether lipids, which is probably advantageous for membrane function. Definitely, more research is necessary to understand and quantify the role of acetogenesis for AMMP. A second explanation for the relative increase of aceticlastic methanogenesis with decreasing temperature is the thermodynamics of H_2 production. Production of H_2 by syntrophic fatty acid oxidation (function 4) becomes increasingly less exergonic when temperature decreases, resulting in less H_2 production (Chin and Conrad, 1995). Both adaptation of acetogens and thermodynamics of syntrophic fatty acid oxidation may result in production of relatively more acetate than H_2 and thus enhancing aceticlastic methanogenesis (Fig. 1B).

The microbial community structures responsible for AMMP are quite complex and diverse. There are differences in the various methanogenic environments, such as rice field soil, lake sediments, acidic peat, animal guts, anaerobic digestors, landfills, saline environments, etc.. Detailed description of the archaeal and bacterial sequences and operational taxonomic units are out of scope for this minireview. Briefly, the community of methanogenic archaea usually contains both putatively hydrogenotrophic taxa (*Methanomicrobiales, Methanobacteriales, Methanocellales, Methanosarcinales*) and putatively aceticlastic taxa (*Methanosarcinaceae, Methanotrichaceae* (formerly *Methanosaetaceae*, (Oren, 2014)), so that both hydrogenotrophic and aceticlastic methanogenesis is possible. An important exception, however, is methanogenesis at elevated temperature (40-50°C). Rice field soils and other anoxic environments with methanogenic activity are frequently able to produce CH_4 under moderately thermophilic conditions (Schulz et al., 1997; Fey et al., 2001). There are soils that contain moderately thermophilic aceticlastic methanogens (Wu et al., 2006). In such a soil, AMMP does not change with temperature increase above 40°C (Liu et al., 2018) and both aceticlastic and hydrogenotrophic methanogenesis operate at the common ratio (Fig. 1A). However, there are other soils in which populations of aceticlastic methanogens vanish when they are exposed to elevated temperatures. These soils then produce CH_4 exclusively by hydrogenotrophic methanogenesis (Conrad et al., 2009; Liu et al., 2018). This is possible, since thermophilic

syntrophic acetate oxidizers convert the initially produced acetate to H_2 plus CO_2 followed by hydrogenotrophic methanogenesis (Liu and Conrad, 2010). Syntrophic acetate oxidation is thermodynamically endergonic under standard conditions, but becomes increasingly less endergonic if temperature increases (Lee and Zinder, 1988). Syntrophic acetate oxidation is the only possibility to remove the intermittently accumulating acetate, which otherwise could not be degraded (Fig. 1C). The syntrophic acetate oxidizers were identified belonging to *Thermoanaerobacteraceae*, and possibly also to *Heliobacteriaceae* (Liu and Conrad, 2010; Liu et al., 2018; Peng et al., 2018).

In principle such syntrophic acetate oxidizers may also operate under mesophilic conditions, especially since mesophilic syntrophic species (e.g., *Clostridium ultunense*; *Syntrophaceticus schinkii*) have been isolated (Schnürer et al., 1996; Westerholm et al., 2010). Indeed contribution of acetate oxidizers to AMMP has been found in some environments with low or moderate temperatures (Chauhan and Ogram, 2006; Nüsslein et al., 2001; Vavilin et al., 2017). The importance of syntrophic acetate oxidizers is obvious, if aceticlastic methanogens (e.g. *Methanotrichaceae*) are lacking (Nüsslein et al., 2001). However, if a methanogenic environment does contain putatively aceticlastic methanogens (which is common for most environments under mesophilic conditions), it is hard to understand why syntrophic acetate oxidation instead of aceticlastic methanogenesis should be operating. One reason may be when the existing aceticlastic methanogens are inhibited by environmental factors. For example, elevated concentrations of ammonia (Schnürer et al., 1999; Zhang et al., 2014; Müller et al., 2016), phosphate (Conrad et al., 2000; Chin et al., 2004), or acetate (Karakashev et al., 2006; Petersen and Ahring, 1991) can be such factors, which inhibit the extant populations of aceticlastic methanogens. Then, acetate would be no longer degraded, or would be incompletely degraded, unless syntrophic acetate oxidizers are active (Fig. 1C). However, our knowledge of syntrophic acetate oxidation is still very low.

Regulation by quality of organic matter

When cellulose is degraded by AMMP, CH_4 is produced by >67% aceticlastic and <33% hydrogenotrophic methanogenesis (Fig. 1A). However, if cellulose is replaced by chitin, which is an important structural material of fungi and arthropods, there is an additional acetate produced from each monomer (i.e., N-acetylglucosamine). Then, the contribution of aceticlastic methanogenesis increases to 75% (or 100% in case fermentation is by acetogenesis).

By contrast, the relative contribution of hydrogenotrophic methanogenesis increases if the organic material is more reduced than saccharides, e.g. lipids. For simplicity, let us assume degradation by AMMP of an alkane hydrocarbon, e.g. hexane:

$C_6H_{14} + 6\ H_2O$	→	$3\ CH_3COOH + 7\ H_2$	(5)
$3\ CH_3COOH$	→	$3\ CH_4 + 3\ CO_2$	(2)
$7\ H_2 + 1.75\ CO_2$	→	$1.75\ CH_4 + 3.5\ H_2O$	(3)
$C_6H_{14} + 2.5\ H_2O$	→	$4.75\ CH_4 + 1.25\ CO_2$	(6)

In degradation of hexane compared to cellulose, AMMP would result in the production of much more CH_4 than CO_2. However, AMMP would still be dominated (67%) by aceticlastic methanogenesis. The stoichiometries above show that lipids or other reduced organic compounds would only marginally affect the methanogenic pathways, which would still be close to that shown for cellulose degradation in Fig.1A. Hence, it is reasonable to conclude that CH_4 production occurs predominantly (>67%) by aceticlastic methanogesis, provided the original organic material is degraded to completion and syntrophic acetate oxidation is negligible.

Note, however, that the emphasis has so far been on the complete degradation of organic matter. If we assume, by contrast, that organic matter is not completely degraded,

stoichiometries may be completely different. Simple aromatic compounds, such as phenylacetate, may be degraded incompletely yielding benzoate, CO_2 and H_2, but yielding no acetate (Harwood et al., 1998):

$$C_8H_7O_2^- + 2\ H_2O \rightarrow C_7H_5O_2^- + CO_2 + 3\ H_2 \quad (7)$$
$$3\ H_2 + 0.75\ CO_2 \rightarrow 0.75\ CH_4 + 1.5\ H_2O \quad (3)$$
$$C_8H_7O_2^- + 0.5\ H_2O \rightarrow C_7H_5O_2^- + 0.75\ CH_4 + 0.25\ CO_2 \quad (8)$$

Such degradation process produces CH_4 exclusively by hydrogenotrophic methanogenesis. Phenylacetate (and phenylpropionate) is indeed a minor product of the AMMP from rice straw (Glissmann et al., 2005) and peat (Kotsyurbenko et al., 2004). More importantly, however, there are plenty of other complex organic substances in soil or sediment that might possibly be degraded in a similar way, thus greatly enhancing the contribution of hydrogenotrophic methanogenesis to AMMP (Fig. 1D). Such processes likely explain the observation that CH_4 production in many lake sediments is produced by the contribution of >50% hydrogenotrophic methanogenesis (Conrad et al., 2010; Conrad et al., 2011; Ji et al., 2016). Lake sediments or accumulating peat layers are likely sites for such processes, since they are formed by a sedimentation process and are not much disturbed. There, only the most labile part of the freshly settled organic matter is completely degraded, while the less labile one accumulates in sediment layers that increase in age with depth (Fig. 1D). Hence, deeper layers of organic matter are increasingly recalcitrant for degradation and support increasingly less CH_4 production, albeit with an increasing contribution of hydrogenotrophic methanogenesis (Conrad et al., 2010; Liu et al., 2017; Vaughn et al., 2016; Hodgkins et al., 2014; Chan et al., 2005).

Rice field soils, by contrast, are regularly ploughed and fresh input of rice straw is degraded by 80-90% in the first season (Neue and Scharpenseel, 1987). In such environment, most of the AMMP occurs by the normal mixture of aceticlastic and hydrogenotrophic methanogenesis (Fig. 1A). Interestingly, even under these conditions does the relative contribution of hydrogenotrophic methanogenesis increase when most (70%) of the rice straw has been degraded, indicating that increasing recalcitrance of organic matter results in increasing contribution of hydrogenotrophic methanogenesis (Ji et al., 2018a).

The methanogenic archaeal communities of environments with incomplete conversion of organic substances and enhanced hydrogenotrophic methanogenesis generally consist of both hydrogenotrophic and aceticlastic methanogenic archaea, albeit hydrogenotrophic ones may be enhanced relatively to the aceticlastic ones (Chan et al., 2005; Liu et al., 2017; Mondav et al., 2014). However, the coexistence of both indicates that the regulation of the pathway of methanogenesis mainly occurs by flux control rather than by control of the functional methanogenic populations.

Alternative fates of methane precursors

If AMMP proceeds as theoretically expected (compare Fig. 1A), the ratio of hydrogenotrophic and aceticlastic methanogenesis may nevertheless change if either H_2 or acetate are consumed or otherwise diverted before they are converted by methanogens (Fig. 1E). This typically happens in animal guts, where acetate (and other short-chain fatty acids) is resorbed as a food source, so that methanogens are left with H_2 as methane precursor (Hungate, 1967). Similarly, acetate may be consumed in microbial or algal mats as a carbon source, so that the contribution of hydrogenotrophic methanogenesis increases (Sandbeck and Ward, 1981). Alternatively, H_2 production and hydrogenotrophic methanogenesis may be stimulated by light-driven processes (Lee et al., 2014). Vice versa, H_2 may be lost from the environment thus enhancing the contribution of aceticlastic methanogenesis. Since H_2 is a gas with low solubility, it may easily evade, especially if it is produced close to the soil-atmosphere interface,

such as in microbial mats (Hoffmann et al., 2015) or is ventilated by ebullition or plant gas vascular systems (Schütz et al., 1988).

The ratio of hydrogenotrophic and aceticlastic methanogenesis may also be affected by temporal change in ecosystem function, such as reported for some northern peatlands. There, CH_4 was predominantly produced by hydrogenotrophic methanogenesis while acetate was not consumed at all and accumulated during the season with high water table (Duddleston et al., 2002)(Fig. 1E). This was due to a lack of aceticlastic methanogens (Rooney-Varga et al., 2007). However, when the water table dropped at the end of season so that O_2 could penetrate into the formerly methanogenic peat layers, acetate was oxidized by microorganisms using O_2 or ferric iron as oxidants (Duddleston et al., 2002). Such temporal changes in environmental conditions may occur in many different ecosystems, e.g., upon water management in rice fields (Krüger et al., 2002; Zhang et al., 2013), so that alternative fates of methane precursors can change the pathway of CH_4 production.

In particular, it may be important to consider the temporary or local effects of inorganic oxidants (O_2, nitrate, sulfate, ferric iron, etc.), which allow oxidation of methanogenic substrates to CO_2 instead of reduction to CH_4 (Fig. 1F). Such oxidation reactions may be selective for either H_2 (such as in Fig. 1F) or acetate and thus, specifically suppress hydrogenotrophic or aceticlastic methanogenesis (Klüber and Conrad, 1998; Achtnich et al., 1995; Conrad, 1999; Scheid et al., 2003). While inorganic oxidants are normally unavailable if methanogenic environments have been under reducing conditions for several days or weeks, they can be relevant electron acceptors in environments with local or temporal input of oxygen, for example provided by plant ventilation or water table fluctuations (Beckmann and Lloyd, 2001; Knorr and Blodau, 2009). Inorganic oxidants (e.g., sulfate) can be plentiful in marine and other saline environments, in which AMMP is a niche process compared to microbial sulfate reduction (Mountfort et al., 1980; Crill and Martens, 1986). There, AMMP may be restricted to the degradation of compatible solutes coupled to methylotrophic methanogenesis (see below).

Regulation by pH

For complete degradation of organic matter in the absence of inorganic oxidants AMMP must work at any pH. The theoretical constraints for complete degradation are the same as outlined above. However, it is feasible that degradation of organic matter is incomplete at pH values that largely deviate from neutrality. The coincidence of accumulating peat layers and acid pH values is obvious. Therefore, it has been argued that imbalances in microbial fermentation processes may contribute to acidification (Zeikus, 1983). Indeed anaerobic H_2 turnover was found to decrease with decreasing pH of lake sediments (Goodwin et al., 1988) indicating that hydrogenotrophic methanogenesis may become increasingly substrate limited. Although aceticlastic methanogenesis is sometimes the almost exclusive CH_4 production process in acidic lake sediments (Goodwin and Zeikus, 1987; Phelps and Zeikus, 1984), this may also be explained by acetogenic fermentation (Fig. 1B). In fact, most studies found the existence of both hydrogenotrophic and aceticlastic methanogenic archaea in sediments of acidic lakes, rivers, peat mires or soils (Horn et al., 2003; Chan et al., 2002; Sanz et al., 2011; Kotsyurbenko et al., 2004; Metje and Frenzel, 2007; Barbier et al., 2012; Liebner et al., 2015; Cadillo-Quiroz et al., 2006; Basiliko et al., 2003). However, aceticlastic methanogens may be absent or inactive in some peat environments resulting in accumulation of acetate (Metje and Frenzel, 2005; Duddleston et al., 2002). With these exceptions, AMMP in acidic environments is not basically different from AMMP in neutral ones. Hydrogenotrophic methanogenesis, in particular, is apparently not impeded by low pH. In methanogenic rice field soils CH_4 production decreases if the soil pH is artificially decreased (Wang et al., 1993; Jugsujinda et al., 1995). It is well known that CH_4 production in acid sulfate soils is strongly suppressed, albeit it is not clear whether this suppression is mainly due to low pH or high concentrations of sulfate

and ferric iron, which may serve as alternative electron acceptors in the anaerobic degradation organic matter (Yao et al., 1999).

Methanogenesis can be found in alkaline environments, which usually are haloalkaline (Sorokin et al., 2015a; Oremland et al., 1982; Antony et al., 2012). The active methanogens apparently belong to the group of methylotrophic methanogens, which are commonly found in saline environments and use so-called "non-competitive" methanogenic substrates such as methanol, methylamines and methyl sulfides (see below). However, hydrogenotrophic alkaliphilic methanogens have also been isolated (Sorokin et al., 2015b).

In summary, there is presently no evidence that pH by itself regulates the relative contribution of hydrogenotrophic versus aceticlastic methanogenesis in terrestrial envrionments. It is probably rather the presence of alternative oxidants (e.g., sulfate in acid precipitation (Gauci et al., 2008)) that affects methanogenesis.

Other CO_2-type methanogenesis

The so-called hydrogenotrophic pathway does not necessarily depend on the provision of H_2 as electron donor. Many hydrogenotrophic methanogens can as well utilize formate, CO or even alcohols (ethanol, 2-propanol, etc.) as substrates (Widdel, 1986; Daniels et al., 1977). Ethanol (and other alcohols) is only partially oxidized resulting in the production of acetate, which is subsequently utilized by aceticlastic methanogens (Fig. 2A). Formate and CO are oxidized to CO_2, while the electrons are used to reduce CO_2 to CH_4. Therefore, all these physiological types can be summarized as CO_2-type methanogens, contrasting acetate-type methanogens and methylated C1 compounds-type methanogens (Liu and Whitman, 2008). Ethanol and 2-propanol, for example can be important methanogenic substrates in peat (Metje and Frenzel, 2005) and pond sediments (Martins et al., 2017). It is not easy to distinguish direct utilization by an alcohol-oxidizing methanogen and syntrophic alcohol oxidation coupled to hydrogenotrophic methanogenesis (Fig. 2A). For the sake of differentiating the hydrogenotrophic methanogenic pathway from aceticlastic and methylotrophic pathways, it actually does not matter how CO_2 is reduced, since alcohols (or formate, CO) are like H_2 normal intermediates in fermentative AMMP, and are summarily included in equation (1). For the differentiation of hydrogenotrophic versus aceticlastic methanogenic pathways it also does not matter whether the methanogens consume the fermentatively produced H_2 in dissolved form, by interspecies-H_2-transfer (or interspecies-formate-transfer), or by direct interspecies electron transfer (DIET) (Bryant et al., 1967; Conrad et al., 1985; Thiele and Zeikus, 1988; Rotaru et al., 2012).

Fig. 2

Fig. 2: Schemes of anaerobic microbial methane production (AMMP) from organic matter (e.g., polysaccharides) by involving the following additional functions: (A) methanogenic conversion of alcohols (function 6); (B) methanogenesis from glycolysis (function 7).

Another intriguing idea would be the existence of glycolytic methanogens, i.e. a methanogenic species, which can utilize glucose as substrate and convert it to CH_4 and CO_2, as shown in equation (4). Thus, the microbial community accomplishing AMMP would be replaced by only one organism, which would do everything (Fig. 2B). This idea is presently pure speculation, but it is not completely unlikely, since some methanogens can metabolize glycogen, which they produce as storage material. For example, *Methanosarcina activorans* displays vigorous gluconeogenesis and glycolysis (Santiago-Martinez et al., 2016). Metatranscriptome analyses of methanogenic paddy soil showed that *Methanosarcinaceae* transcribe glucosyl hydrolases involved in polymer breakdown (Peng et al., 2018). Hence, why should species of methanogens not be able to utilize glucose or polysaccharides entirely without sharing the energy content with other members of a complex microbial community? Theory

suggests that microbes can optimize either rate or yield of ATP production (Pfeiffer et al., 2001). While high ATP yields would favor complete degradation (glucose to CH_4), high ATP rates are achieved by reducing the length of the pathway (e.g, acetate to CH_4). Our procedures for enrichment and isolation of microbes are all selecting for fast growing species. However, slow growing species with optimized ATP yields may be found in biofilms or aggregates (Kreft, 2004). Such microbes may have not yet been isolated because of technical reasons. The completely nitrifying bacteria (Comammox), which have recently been isolated and described (Kits et al., 2017), may be considered as an analogue for putatively glycolytic methanogens. Both accomplish complete conversion of a substrate, which is normally converted by a chain of processes divided to several different microbes. However, existence of putatively glycolytic methanogens would result in the same stoichiometry of polysaccharide degradation as shown in equations (1-4), albeit being catalyzed in one organism instead of a community (Fig. 2B). The percentage of 33% hydrogenotrophic and 67% aceticlastic methanogenesis would be the same, since glycolysis would eventually result in the production of 2 acetyl-CoA and 4 hydrogen equivalents, analogous to equation (1) and these glycolytic products would be converted by the methanogenic machinery to CH_4 analogous to equations (2) and (3).

Role of methylotrophic methanogenesis

Whereas aceticlastic and hydrogenotrophic methanogens are required for AMMP in almost all anoxic methanogenic environments, the role of methylotrophic methanogenesis is not completely clear. Methylotrophic methanogens all belong to the family *Methanosarcinaceae*, except the genus *Methanosphaera*, which belongs to the order *Methanobacteriales* (Liu and Whitman, 2008). Methylotrophic methanogenesis is the conversion of a methyl compound to CH_4 plus CO_2, e.g. methanol, which is disproportionated to CH_4 and CO_2:

$$4\ CH_3OH \rightarrow CO_2 + 2\ H_2O + 3\ CH_4 \quad (9)$$

Methanol is a product of pectin degradation and can be degraded by methanogens (Schink and Zeikus, 1980). However, pectin turnover in lake sediment is only slow (Schink and Zeikus, 1982), and methanol has been found to be only a marginal methanogenic precursor (Lovley and Klug, 1983). The same is true for anoxic rice field soil (Conrad and Claus, 2005). Since small amounts of methanol may also be produced from xylan (Rosell and Svensson, 1975) and lignin (Ander et al., 1985), methanol-driven methanogenesis cannot be completely dismissed (Fig. 3A). However, it may primarily be important for special environments, such as methanogenic wetwood, where wood pectin is degraded by AMMP (Schink et al., 1981). Peat soil from the Zoige wetlands was also claimed to produce CH_4 mainly from methanol (Jiang et al., 2010). However, even if pectin (or xylan) is the major polymer driving methanogenesis, AMMP would not only result in the production of methanol, but even more in the production of monosaccharides (e.g., gluconic acid, galacturonic acid) that are further degraded to acetate, CO_2 and H_2. Each of these sugar acid molecules would result in the production of two molecules of acetate, which are methanogenically converted to two molecules of CH_4. Hence, pectin would stimulate methanogenesis from acetate more from methanol (Fig. 3A).

Fig. 3

Fig. 3 Schemes of anaerobic microbial methane production (AMMP) from organic matter (e.g., pectin, xylan) involving methylotrophic methanogenesis (function 8): (A) General degradation scheme involving CH_4 production from methanol and trimethylamine (TMA); (B) methanol-dependent methanogenesis and resorption of acetate (e.g., in gut systems); (C) TMA-dependent methanogenesis and predominant utilization of acetate and H_2 by sulfate reduction.

More recently, methanol-degrading methanogens of the order *Methanomassiliicoccales* have been described (Paul et al., 2012; Dridi et al., 2012). These methanogens reduce methanol with H_2:

$$CH_3OH + H_2 \rightarrow CH_4 + H_2O \quad (10)$$

These methanogens occur in animal gut systems, but have also frequently been detected in other anoxic environments such as fens (Söllinger et al., 2016), mangrove sediments (Li et al., 2016), digesters (Wilkins et al., 2015), and anoxic paddy soil (Ji et al., 2018b). *Methanomassiliicoccales* are probably even more widespread, since they have only recently been added to sequence data bases and up to then have probably been recorded as *Thermoplasmatales*-related Euryarchaeota. Their role in carbon flux is presently unknown. In gut systems they may play an important role, since acetate is resorbed thus rendering methanol a relatively important methanogenic precursor (Fig. 3B). However, the relative contribution of methylotrophic versus hydrogenotrophic methanogenesis in gut systems is presently not known (Söllinger et al., 2018). The genus *Methanosphaera* can also reduce methanol with H_2 to CH_4 (Liu and Whitman, 2008). Recently, genomic information indicate that this type of physiology may also occur in several archaeal candidate taxa, e.g. *Verstraetearchaeota* (Vanwonterghem et al., 2016).

Methylamines, trimethylamine (TMA) in particular, may be important methanogenic substrates in saline environments, where biota produce glycine betaine as osmolyte. Betaine is fermented yielding TMA, which is a methanogenic substrate (Hippe et al., 1979; King, 1984a). Since TMA seems to be a substrate that is better degraded by methanogens than by sulfate reducers, TMA is a so-called non-competitive methanogenic substrate (Oremland and Polcin, 1982; King, 1984b). Thus, it was found that methylotrophic methanogenesis is responsible for much of the CH_4 production in anoxic marine environments despite the presence of active sulfate reducers. These sulfate reducers were successfully competing for H_2 and acetate, but not for methyl compounds like TMA, which was exclusively degraded by methanogens (Fig. 3C). Similarly, CH_4 production seems to be caused by methylotrophic methanogens in many saline environments, e.g. saline lake sediments on the Tibetan Plateau (Liu et al., 2016), hypersaline microbial mats (Smith et al., 2008), and other hypersaline environments (Oren, 1990; Zhuang et al., 2016; Sorokin et al., 2017).

Methanethiol or dimethylsulfide can also serve as methanogenic substrates (Zinder and Brock, 1978; Ni and Boone, 1991; Lomans et al., 1999). Like methylamines, these methylsulfides are produced from the osmolyte dimethyl sulfoniopropionate (Kiene and Visscher, 1987). Dimethyl sulfide may serve a similar role as trimethylamine. However, degradation of dimethyl sulfide happens not only in marine (Zhuang et al., 2018; VanderMaarel and Hansen, 1997) but also in freshwater environments (Lomans et al., 1997).

Conclusions and outlook

Anaerobic degradation of organic matter by soil microbial communities eventually results in the production of CH_4 by aceticlastic and hydrogenotrophic methanogenesis. These two major pathways of microbial CH_4 production may contribute to various percentages ranging from zero to 100%. The extent of their contributions depend on whether the available organic matter is degraded more or less completely (such as straw in paddy fields) or is only partially degraded (such as soil or sediment organic matter). The complete degradation of organic matter is tightly constrained by the stoichiometry of the fermentation reactions, which result in the production of acetate and H_2 at a ratio that consequently gives rise to CH_4 production by >67% aceticlastic and <33% hydrogenotrophic methanogenesis. This ratio is similar for various organic substrates, provided they are completely degraded and the fermentation products are exclusively consumed by fermentation and methanogenesis, and are not accumulated, absorbed,

volatilized or consumed by oxidation reactions coupled to the reduction of inorganic electron acceptors such as ferric iron or sulfate. However, since environmental conditions affect the thermodynamics and kinetics of the individual reactions, and also affect population dynamics and the structuring of the entire microbial community, fermentation reactions can be pushed into production of surplus acetate or H_2. Thus, temperature can control the methanogenic pathways by influencing the thermodynamics of fermentative production of H_2 and acetate, and by affecting the composition of the microbial communities. While acetogenesis followed by aceticlastic methanogenesis is favored at low temperature, syntrophic acetate oxidation followed by hydrogenotrophic methanogenesis is favored at high temperature.

It is remarkable that the temperature control of the methanogenic pathways primarily depends on the activity of acetate-producing and acetate-oxidizing bacteria, which thus play a key role for the regulation of AMMP. The group of acetate-producing microorganisms is termed acetogenic or homoacetogenic bacteria, since they produce acetate as virtually sole fermentation product using the Wood-Ljungdahl pathway (Drake, 1994). Those, which produce acetate chemolithotrophically from H_2 and CO_2 (at least some of them), can reverse the direction of the process if thermodynamic conditions allow and then, oxidize acetate to H_2 plus CO_2 (Zinder, 1994). Under such conditions, they act as syntrophic acetate-oxidizers. Hence, control of the methanogenic pathways depends to a large extent on the activity of acetogens/acetotrophs operating the Wood-Ljungdahl pathway. Unfortunately, this conclusion is primarily based on our knowledge of the regulation of the relative contribution of aceticlastic versus hydrogenotrophic methanogenic pathways, which is indirect evidence. Acetogenic bacteria are widespread in nature and can display remarkable activity, such as in the litter layer of forest soil (Küsel and Drake, 1994), the gut of termites (Breznak and Switzer, 1986), or the deep biosphere (Lever, 2012). However, the quantification of their contribution to the anaerobic degradation of organic matter (e.g., AMMP) in-situ is very difficult and has so far not been achieved unequivocally (Hädrich et al., 2012; Fu et al., 2018; Liu and Conrad, 2011; Hoehler et al., 1999). Understanding the ecology of acetogens/acetotrophs is certainly an important objective for future research.

The role of methylotrophic methanogenesis in nature is also an interesting research question. Methylotrophic methanogenesis is probably important in highly saline environments, where biota form large amounts of compatible solutes that are anaerobically degraded to methyl compounds. Although this conclusion has never been questioned, there are only few studies quantifying the carbon flow under in-situ conditions (King, 1984a).

The role of methanol-dependent methanogenesis under field conditions is completely unclear. Recent studies indicate a remarkable diversity of potentially methanol-reducing methanogens across several archaeal classes (Vanwonterghem et al., 2016; Nobu et al., 2016; Evans et al., 2015). However, to be important for carbon flow, production rates of methanol must be comparable to those of H_2 and acetate to allow significant contribution to total CH_4 production. Since concentrations of methanol are usually below the detection limit, methanol is not likely to play a major role for CH_4 production. However, there are only very few studies addressing methylotrophic methanogenesis to total CH_4 production in non-extreme soil and aquatic environments (Lovley and Klug, 1983; Conrad and Claus, 2005).

ACKNOWLEDGEMENTS

The study was financially supported by the Fonds der Chemischen Industrie, Germany.

References

Achtnich, C., Schuhmann, A., Wind, T. and Conrad, R. (1995) Role of interspecies H_2 transfer to sulfate and ferric iron- reducing bacteria in acetate consumption in anoxic paddy soil. *FEMS Microbiol. Ecol.* 16**,** 61-69.

Ander, P., Eriksson, M. E. R. and Eriksson, K. E. (1985) Methanol production from lignin-related substances by *Phanerochaete chrysosporium*. *Physiol. Plantarum* 65**,** 317-3.

Antony, C. P., Murrell, J. C. and Shouche, Y. S. (2012) Molecular diversity of methanogens and identification of *Methanolobus* sp. as active methylotrophic *Archaea* in Lonar Crater Lake sediments. *FEMS Microbiol. Ecol.* 81**,** 43-51.

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

Basiliko, N., Yavitt, J. B., Dees, P. M. and Merkel, S. M. (2003) Methane biogeochemistry and methanogen communities in two northern peatland ecosystems, New York State. *Geomicrobiol. J.* 20**,** 563-577.

Beckmann, M. and Lloyd, D. (2001) Mass spectrometric monitoring of gases (CO_2, CH_4, O_2) in a mesotrophic peat core from Kopperas Mire, Sweden. *Global Change Biol.* 7**,** 171-180.

Breznak, J. A. and Switzer, J. M. (1986) Acetate synthesis from H2 plus CO2 by termite gut microbes. *Appl. Environ. Microbiol.* 52**,** 623-630.

Bridgham, S. D., Cadillo-Quiroz, H., Keller, J. K. and Zhuang, Q. (2013) Methane emissions from wetlands: biogeochemical, microbial, and modeling perspectives from local to global scales [review]. *Global Change Biology* 19**,** 1325-1346.

Brownlow, R., Lowry, D., Fisher, R. E., France, J. L., Lanoiselle, M., White, B. et al. (2017) Isotopic ratios of tropical methane emissions by atmospheric measurement. *Global Biogeochem. Cycles* 31**,** 1408-1419.

Bryant, M. P., Wolin, E. A., Wolin, M. J. and Wolfe, R. S. (1967) Methanobacillus omelianskii, a symbiotic association of two species of bacteria. *Arch. Mikrobiol.* 59**,** 20-31.

Cadillo-Quiroz, H., Bräuer, S., Yashiro, E., Sun, C., Yavitt, J. and Zinder, S. (2006) Vertical profiles of methanogenesis and methanogens in two contrasting acidic peatlands in central New York State, USA. *Environ. Microbiol.* 8**,** 1428-1440.

Cappenberg, T. and Prins, R. A. (1974) Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a fresh-water lake. III. Experiments with 14C-labeled substrates. *Ant. Leeuwenhoek* 40**,** 457-469.

Chan, O. C., Claus, P., Casper, P., Ulrich, A., Lueders, T. and Conrad, R. (2005) Vertical distribution of structure and function of the methanogenic archaeal community in Lake Dagow sediment. *Environ. Microbiol.* 7**,** 1139-1149.

Chan, O. C., Wolf, M., Hepperle, D. and Casper, P. (2002) Methanogenic archaeal community in the sediment of an artificially partitioned acidic bog lake. *FEMS Microbiol. Ecol.* 42**,** 119-129.

Chauhan, A. and Ogram, A. (2006) Phylogeny of acetate-utilizing microorganisms in soils along a nutrient gradient in the Florida everglades. *Appl. Environ. Microbiol.* 72**,** 6837-6840.

Chin, K. J. and Conrad, R. (1995) Intermediary metabolism in methanogenic paddy soil and the influence of temperature. *FEMS Microbiol. Ecol.* 18**,** 85-102.

Chin, K. J., Lueders, T., Friedrich, M. W., Klose, M. and Conrad, R. (2004) Archaeal community structure and pathway of methane formation on rice roots. *Microb. Ecol.* 47**,** 59-67.

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

Conrad, R. (2009) The global methane cycle: Recent advances in understanding the microbial processes involved [Minireview]. *Environ. Microbiol. Reports* 1**,** 285-292.

Conrad, R., Bak, F., Seitz, H. J., Thebrath, B., Mayer, H. P. and Schütz, H. (1989) Hydrogen turnover by psychrotrophic homoacetogenic and mesophilic methanogenic bacteria in anoxic paddy soil and lake sediment. *FEMS Microbiol. Ecol.* 62**,** 285-294.

Conrad, R. and Claus, P. (2005) Contribution of methanol to the production of methane and its ^{13}C-isotopic signature in anoxic rice field soil. *Biogeochem.* 73**,** 381-393.

Conrad, R., Claus, P. and Casper, P. (2010) Stable isotope fractionation during the methanogenic degradation of organic matter in the sediment of an acidic bog lake, Lake Grosse Fuchskuhle. *Limnol. Oceanogr.* 55**,** 1932-1942.

Conrad, R., Klose, M. and Claus, P. (2000) Phosphate inhibits acetotrophic methanogenesis on rice roots. *Appl. Environ. Microbiol.* 66**,** 828-831.
Conrad, R., Klose, M. and Noll, M. (2009) Functional and structural response of the methanogenic microbial community in rice field soil to temperature change. *Environ. Microbiol.* 11**,** 1844-1853.
Conrad, R., Noll, M., Claus, P., Klose, M., Bastos, W. R. and Enrich-Prast, A. (2011) Stable carbon isotope discrimination and microbiology of methane formation in tropical anoxic lake sediments. *Biogeosciences* 8**,** 795-814.
Conrad, R., Phelps, T. J. and Zeikus, J. G. (1985) Gas metabolism evidence in support of juxtapositioning between hydrogen producing and methanogenic bacteria in sewage sludge and lake sediments. *Appl. Environ. Microbiol.* 50**,** 595-601.
Conrad, R. and Wetter, B. (1990) Influence of temperature on energetics of hydrogen metabolism in homoacetogenic, methanogenic, and other anaerobic bacteria. *Arch. Microbiol.* 155**,** 94-98.
Crill, P. M. and Martens, C. S. (1986) Methane production from bicarbonate and acetate in an anoxic marine sediment. *Geochim. Cosmochim. Acta* 50**,** 2089-2097.
Daniels, L., Fuchs, G., Thauer, R. K. and Zeikus, J. G. (1977) Carbon monoxide oxidation by methanogenic bacteria. *J. Bacteriol.* 132**,** 118-126.
Drake, H. L. (1994) Acetogenesis, acetogenic bacteria, and the acetyl-CoA "Wood/Ljungdahl" pathway: past and current perspectives. In *Acetogenesis*. Drake, H. L. (ed). New York: Chapman & Hall, pp. 3-60.
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.
Duddleston, K. N., Kinney, M. A., Kiene, R. P. and Hines, M. E. (2002) Anaerobic microbial biogeochemistry in a northern bog: Acetate as a dominant metabolic end product. *Global Biogeochem. Cycles* 16**,** 1063-doi:10.1029/2001GB001402.
Duin, E. C., Wagner, T., Shima, S., Prakash, D., Cronin, B., Yanez-Ruiz, D. R. et al. (2016) Mode of action uncovered for the specific reduction of methane emissions from ruminants by the small molecule 3-nitrooxypropanol. *Proc. Natl. Acad. Sci. USA* 113**,** 6172-6177.
Ehhalt, D. H. (1979) Der atmosphärische Kreislauf von Methan. *Naturwiss.* 66**,** 307-311.
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.
Fey, A., Chin, K. J. and Conrad, R. (2001) Thermophilic methanogens in rice field soil. *Environ. Microbiol.* 3**,** 295-303.
Fey, A. and Conrad, R. (2000) Effect of temperature on carbon and electron flow and on the archaeal community in methanogenic rice field soil. *Appl. Environ. Microbiol.* 66**,** 4790-4797.
Fu, B., Conrad, R. and Blaser, M. (2018) Potential contribution of acetogenesis to anaerobic degradation in methanogenic rice field soils. *Soil Biol. Biochem.* 119**,** 1-10.
Gauci, V., Dise, N. B., Howell, G. and Jenkins, M. E. (2008) Suppression of rice methane emission by sulfate deposition in simulated acid rain. *J. Geophys. Res. -Biogeosci.* 113**,** G00A7-doi:10.1029/2007JG000501.
Glissmann, K., Chin, K. J., Casper, P. and Conrad, R. (2004) Methanogenic pathway and archaeal community structure in the sediment of eutrophic Lake Dagow: Effect of temperature. *Microb. Ecol.* 48**,** 389-399.
Glissmann, K., Hammer, E. and Conrad, R. (2005) Production of aromatic compounds during methanogenic degradation of straw in rice field soil. *FEMS Microbiol. Ecol.* 52**,** 43-48.
Goodwin, S., Conrad, R. and Zeikus, J. G. (1988) Influence of pH on microbial hydrogen metabolism in diverse sedimentary ecosystems. *Appl. Environ. Microbiol.* 54**,** 590-593.
Goodwin, S. and Zeikus, J. G. (1987) Ecophysiological adaptations of anaerobic bacteria to low pH: analysis of anaerobic digestion in acidic bog sediments. *Appl. Environ. Microbiol.* 53**,** 57-64.
Hackstein, J. H. P., Langer, P. and Rosenberg, J. (1996) Genetic and evolutionary constraints for the symbiosis between animals and methanogenic bacteria. *Environ. Monitoring and Assessment* 42**,** 39-56.
Hädrich, A., Heuer, V. B., Herrmann, M., Hinrichs, K. U. and Küsel, K. (2012) Origin and fate of acetate in an acidic fen. *FEMS Microbiol. Ecol.* 81**,** 339-354.

Harwood, C. S., Burchhardt, G., Herrmann, H. and Fuchs, G. (1998) Anaerobic metabolism of aromatic compounds via the benzoyl-CoA pathway. *FEMS Microbiol. Rev.* 22**,** 439-458.

Hippe, H., Caspari, D., Fiebig, K. and Gottschalk, G. (1979) Utilization of trimethylamine and other N-methyl compounds for growth and methane formation by Methanosarcina barkeri. *Proc. Natl. Acad. Sci. USA* 76**,** 494-498.

Hodgkins, S. B., Tfaily, M. M., McCalley, C. K., Logan, T. A., Crill, P. M., Saleska, S. R. et al. (2014) Changes in peat chemistry associated with permafrost thaw increase greenhouse gas production. *Proc. Natl. Acad. Sci. USA* 111**,** 5819-5824.

Hoehler, T. M., Albert, D. B., Alperin, M. J. and Martens, C. S. (1999) Acetogenesis from CO_2 in an anoxic marine sediment. *Limnol. Oceanogr.* 44**,** 662-667.

Hoffmann, D., Maldonado, J., Wojciechowski, M. F. and Garcia-Pichel, F. (2015) Hydrogen export from intertidal cyanobacterial mats: sources, fluxes and the influence of community composition. *Environ. Microbiol.* 17**,** 3738-3753.

Holland, H. D., Lazar, B. and McCaffrey, M. (1986) Evolution of the atmosphere and oceans. *Nature* 320**,** 27-33.

Horn, M. A., Matthies, C., Küsel, K., Schramm, A. and Drake, H. L. (2003) Hydrogenotrophic methanogenesis by moderately acid-tolerant methanogens of a methane-emitting acidic peat. *Appl. Environ. Microbiol.* 69**,** 74-83.

Hristov, A. N., Oh, J., Giallongo, F., Frederick, T. W., Harper, M. T., Weeks, H. L. et al. (2015) An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. *Proc. Natl. Acad. Sci. USA* 112**,** 10663-10668.

Hungate, R. E. (1967) Hydrogen as an intermediate in the rumen fermentation. *Arch. Mikrobiol.* 59**,** 158-164.

Ji, Y., Angel, R., Klose, M., Claus, P., Marotta, H., Pinho, L. et al. (2016) Structure and function of methanogenic microbial communities in sediments of Amazonian lakes with different water types. *Environ. Microbiol.* 18**,** 5082-5100.

Ji, Y., Liu, P. and Conrad, R. (2018a) Change of the pathway of methane production with progressing anoxic incubation of paddy soil. *Soil Biol. Biochem.* 121**,** 177-184.

Ji, Y., Liu, P. and Conrad, R. (2018b) Response of fermenting bacterial and methanogenic archaeal communities in paddy soil to progressing rice straw degradation. *Soil Biol. Biochem.* 124**,** 70-80.

Jiang, N., Wang, Y. F. and Dong, X. Z. (2010) Methanol as the primary methanogenic and acetogenic precursor in the cold Zoige Wetland at Tibetan Plateau. *Microb. Ecol.* 60**,** 206-213.

Jugsujinda, A., DeLaune, R. D., Lindau, C. W., Sulaeman, E. and Pezeshki, S. R. (1995) Factors controlling carbon dioxide and methane production in acid sulfate soils. *Water Air and Soil Pollution* 84**,** 1-11.

Karakashev, D., Batstone, D. J., Trably, E. and Angelidaki, I. (2006) Acetate oxidation is the dominant methanogenic pathway from acetate in the absence of *Methanosaetaceae*. *Appl. Environ. Microbiol.* 72**,** 5138-5141.

Kiene, R. P. and Visscher, P. T. (1987) Production and fate of methylated sulfur compounds from methionine and dimethylsulfoniopropionate in anoxic salt marsh sediments. *Appl. Environ. Microbiol.* 53**,** 2426-2434.

King, G. M. (1984a) Metabolism of trimethylamine, choline, and glycine betaine by sulfate-reducing and methanogenic bacteria in marine sediments. *Appl. Environ. Microbiol.* 48**,** 719-725.

King, G. M. (1984b) Utilization of hydrogen, acetate, and "noncompetitive" substrates by methanogenic bacteria in marine sediments. *Geomicrobiol. J.* 3**,** 275-306.

Kits, K. D., Sedlacek, C. J., Lebedeva, E. V., Han, P., Bulaev, A., Pjevac, P. et al. (2017) Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle. *Nature* 549**,** 269-272.

Klüber, H. D. and Conrad, R. (1998) Inhibitory effects of nitrate, nitrite, NO and N_2O on methanogenesis by *Methanosarcina barkeri* and *Methanobacterium bryantii*. *FEMS Microbiol. Ecol.* 25**,** 331-339.

Knorr, K. H. and Blodau, C. (2009) Impact of experimental drought and rewetting on redox transformations and methanogenesis in mesocosms of a northern fen soil. *Soil Biol. Biochem.* 41**,** 1187-1198.

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

Kotsyurbenko, O. R., Glagolev, M. V., Nozhevnikova, A. N. and Conrad, R. (2001) Competition between homoacetogenic bacteria and methanogenic archaea for hydrogen at low temperature. *FEMS Microbiol. Ecol.* 38**,** 153-159.

Kreft, J. U. (2004) Biofilms promote altruism. *Microbiology-UK* 150**,** 2751-2760.

Krüger, M., Eller, G., Conrad, R. and Frenzel, P. (2002) Seasonal variation in pathways of CH_4 production and in CH_4 oxidation in rice fields determined by stable carbon isotopes and specific inhibitors. *Global Change Biol.* 8**,** 265-280.

Küsel, K. and Drake, H. L. (1994) Acetate synthesis in soil from a Bavarian beech forest. *Appl. Environ. Microbiol.* 60**,** 1370-1373.

Lee, J. Z., Burow, L. C., Woebken, D., Everroad, R., Kubo, M. D., Spormann, A. M. et al. (2014) Fermentation couples Chloroflexi and sulfate-reducing bacteria to cyanobacteria in hypersaline microbial mats. *Frontiers Microbiol.* 5**,** 61-doi: 10.3389/fmicb.2014.00061 .

Lee, M. J. and Zinder, S. H. (1988) Hydrogen partial pressures in a thermophilic acetate-oxidizing methanogenic coculture. *Appl. Environ. Microbiol.* 54**,** 1457-1461.

Lever, M. A. (2012) Acetogenesis in the energy-starved deep biosphere - a paradox? *Front. Microbio.* 2**,** 2-doi:10.3389/fmicb.2011.00284.

Li, W., Guan, W., Chen, H., Liao, B. W., Hu, J., Peng, C. H. et al. (2016) Archaeal communities in the sediments of different mangrove stands at Dongzhaigang, China. *J. Soils Sed.* 16**,** 1995-2004.

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

Liu, F. and Conrad, R. (2011) Chemolithotrophic acetogenic H2/CO2 utilization in Italian rice field soil. *ISME J.* 5**,** 1526-1539.

Liu, F. H. and Conrad, R. (2010) *Thermoanaerobacteriaceae* oxidize acetate in methanogenic rice field soil at 50°C. *Environ. Microbiol.* 12**,** 2341-2354.

Liu, P., Klose, M. and Conrad, R. (2018) Temperature effects on structure and function of the methanogenic microbial communities in two paddy soils and one desert soil. *Soil Biol. Biochem.* 124**,** 236-244.

Liu, Y., Conrad, R., Yao, T., Gleixner, G. and Claus, P. (2017) Change of methane production pathway with sediment depth in a lake on the Tibetan plateau. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 474**,** 279-286.

Liu, Y., Priscu, J. C., Xiong, J., Conrad, R., Vick-Majors, T., Chu, H. and Hou, J. (2016) Salinity drives archaeal distribution patterns in high altitude lake sediments on the Tibetan Plateau. *FEMS Microbiol. Ecol.* 92**,** fiw033-doi: 10.1093/femsec/fiw033.

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

Lomans, B. P., Maas, R., Luderer, R., Dencamp, H. J. M. O., Pol, A., VanderDrift, C. and Vogels, G. D. (1999) Isolation and characterization of *Methanomethylovorans hollandica* gen. nov., sp nov., isolated from freshwater sediment, a methylotrophic methanogen able to grow on dimethyl sulfide and methanethiol. *Appl. Environ. Microbiol.* 65**,** 3641-3650.

Lomans, B. P., Smolders, A. J. P., Intven, L. M., Pol, A., Dencamp, H. J. M. O. and VanderDrift, C. (1997) Formation of dimethyl sulfide and methanethiol in anoxic freshwater sediments. *Appl. Environ. Microbiol.* 63**,** 4741-4747.

Lovley, D. R. and Klug, M. J. (1983) Methanogenesis from methanol and methylamines and acetogenesis from hydrogen and carbon dioxide in sediments of a eutrophic lake. *Appl. Environ. Microbiol.* 45**,** 1310-1315.

Martins, P. D., Hoyt, D. W., Bansal, S., Mills, C. T., Tfaily, M., Tangen, B. A. et al. (2017) Abundant carbon substrates drive extremely high sulfate reduction rates and methane fluxes in prairie pothole wetlands. *Global Change Biology* 23**,** 3107-3120.

Metje, M. and Frenzel, P. (2005) Effect of temperature on anaerobic ethanol oxidation and methanogenesis in acidic peat from a northern wetland. *Appl. Environ. Microbiol.* 71**,** 8191-8200.

Metje, M. and Frenzel, P. (2007) Methanogenesis and methanogenic pathways in a peat from subarctic permafrost. *Environ. Microbiol.* 9**,** 954-964.

Mondav, R., Woodcroft, B. J., Kim, E. H., McCalley, C. K., Hodgkins, S. B., Crill, P. M. et al. (2014) Discovery of a novel methanogen prevalent in thawing permafrost. *Nature Commun.* 5**,** 3212-DOI:10.1038/ncomms4212.

Mountfort, D. O., Asher, R. A., Mays, E. L. and Tiedje, J. M. (1980) Carbon and electron flow in mud and sandflat intertidal sediments at Delaware Inlet, Nelson, New Zealand. *Appl. Environ. Microbiol.* 39**,** 686-694.

Müller, B., Sun, L., Westerholm, M. and Schnürer, A. (2016) Bacterial community composition and fhs profiles of low- and high-ammonia biogas digesters reveal novel syntrophic acetate-oxidising bacteria. *Biotechnol. Biofuels* 9**,** 48-doi:10.1186/s13068-016-0454-9.

Neue, H. U. and Scharpenseel, H. W. (1987) Decomposition pattern of 14C-labeled rice straw in aerobic and submerged rice soils of the Philippines. *Sci. Total Environ.* 62**,** 431-434.

Ni, S. and Boone, D. R. (1991) Isolation and characterization of a dimethyl sulfide-degrading methanogen, Methanolobus siciliae HI350, from an oil well, characterization of M.siciliae T4/MT, and emendation of M.siciliae. *Int. J. Syst. Bact.* 41**,** 410-416.

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 methylreducing methanogen. *ISME J.* 10**,** 2478-2487.

Nozhevnikova, A. N., Kotsyurbenko, O. R. and Simankova, M. V. (1994) Acetogenesis at low temperature. In *Acetogenesis*. Drake, H. L. (ed). New York: Chapman & Hall, pp. 416-431.

Nozhevnikova, A. N., Nekrasova, V., Ammann, A., Zehnder, A. J. B., Wehrli, B. and Holliger, C. (2007) Influence of temperature and high acetate concentrations on methanogenensis in lake sediment slurries. *FEMS Microbiol. Ecol.* 62**,** 336-344.

Nüsslein, B., Chin, K. J., Eckert, W. and Conrad, R. (2001) Evidence for anaerobic syntrophic acetate oxidation during methane production in the profundal sediment of subtropical Lake Kinneret (Israel). *Environ. Microbiol.* 3**,** 460-470.

Oremland, R. S., Marsh, L. and DesMarais, D. J. (1982) Methanogenesis in Big Soda Lake, Nevada: an alkaline, moderately hypersaline desert lake. *Appl. Environ. Microbiol.* 43**,** 462-468.

Oremland, R. S. and Polcin, S. (1982) Methanogenesis and sulfate reduction: competitive and noncompetitive substrates in estuarine sediments. *Appl. Environ. Microbiol.* 44**,** 1270-1276.

Oren, A. (1990) Formation and Breakdown of Glycine Betaine and Trimethylamine in Hypersaline Environments. *Ant. Leeuwenhoek* 58**,** 291-298.

Oren, A. (2014) The family *Methanotrichaceae*. In *The Prokaryotes*. Rosenberg, E., DeLong, E. F., Lory, S., Stackebrandt, E. and Thompson, F. (eds). Berlin: Springer, pp. 298-306.

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.

Peng, J., Wegner, C. E., Bei, Q., Liu, P. and Liesack, W. (2018) Metatranscriptomics reveals a differential temperature effect on the structural and functional organization of the anaerobic food web in rice field soil. *Microbiome* 6**,** 169-https://doi.org/10.1186/s40168-018-0546-9.

Petersen, S. P. and Ahring, B. K. (1991) Acetate oxidation in a thermophilic anaerobic sewage-sludge digestor - The importance of non-aceticlastic methanogenesis from acetate. *FEMS Microbiol. Ecol.* 86**,** 149-157.

Pfeiffer, T., Schuster, S. and Bonhoeffer, S. (2001) Cooperation and competition in the evolution of ATP-producing pathways. *Science* 292**,** 504-507.

Phelps, T. J. and Zeikus, J. G. (1984) Influence of pH on terminal carbon metabolism in anoxic sediments from a mildly acidic lake. *Appl. Environ. Microbiol.* 48**,** 1088-1095.

Rooney-Varga, J. N., Giewat, M. W., Duddleston, K. N., Chanton, J. P. and Hines, M. E. (2007) Links between archaeal community structure, vegetation type and methanogenic pathway in Alaskan peatlands. *FEMS Microbiol. Ecol.* 60**,** 240-251.

Rosell, K. G. and Svensson, S. (1975) Studies of the distribution of the 4-O-methyl-D-glucuronic acid residues in birch xylan. *Carbohydrate Res.* 42**,** 297-304.

Rotaru, A. E., Shrestha, P. M., Liu, F., Ueki, T., Nevin, K., Summers, Z. M. and Lovley, D. R. (2012) Interspecies electron transfer via hydrogen and formate rather than direct electrical connections in cocultures of *Pelobacter carbinolicus* and *Geobacter sulfurreducens*. *Appl. Environ. Microbiol.* 78**,** 7645-7651.

Sandbeck, K. A. and Ward, D. M. (1981) Fate of immediate methane precursors in low-sulfate, hot-spring algal-bacterial mats. *Appl. Environ. Microbiol.* 41**,** 775-782.

Santiago-Martinez, M. G., Encalada, R., Lira-Silva, E., Pineda, E., Gallardo-Perez, J. C., Reyes-Garcia, M. A. et al. (2016) The nutritional status of *Methanosarcina acetivorans* regulates glycogen metabolism and gluoneogenesis and glycolysis fluxes. *FEBS J.* 283**,** 1979-1999.

Sanz, J. L., Rodriguez, N., Diaz, E. E. and Amils, R. (2011) Methanogenesis in the sediments of Rio Tinto, an extreme acidic river. *Environ. Microbiol.* 13**,** 2336-2341.

Scheid, D., Stubner, S. and Conrad, R. (2003) Effects of nitrate- and sulfate-amendment on the methanogenic populations in rice root incubations. *FEMS Microbiol. Ecol.* 43**,** 309-315.

Schink, B. and Stams, A. J. M. (2013) Syntrophism among prokaryotes. In *The Prokaryotes: Prokaryotic Communities and Ecophysiology*. Rosenberg, E., DeLong, E. F., Lory, S., Stackebrandt, E. and Thompson, F. (eds). Berlin: Springer, pp. 471-493.

Schink, B., Ward, J. C. and Zeikus, J. G. (1981) Microbiology of wetwood: importance of pectin degradation and Clostridium species in living trees. *Appl. Environ. Microbiol.* 42**,** 526-532.

Schink, B. and Zeikus, J. G. (1980) Microbial methanol formation: A major end product of pectin metabolism. *Curr. Microbiol.* 4**,** 387-389.

Schink, B. and Zeikus, J. G. (1982) Microbial ecology of pectin decomposition in anoxic lake sediments. *J. Gen. Microbiol.* 128**,** 393-404.

Schnürer, A., Schink, B. and Svensson, B. H. (1996) *Clostridium ultunense* sp.nov., a mesophilic bacterium oxidizing acetate in syntrophic association with a hydrogenotrophic methanogenic bacterium. *Int. J. Syst. Bacteriol.* 46**,** 1145-1152.

Schnürer, A., Zellner, G. and Svensson, B. H. (1999) Mesophilic syntrophic acetate oxidation during methane formation in biogas reactors. *FEMS Microbiol. Ecol.* 29**,** 249-261.

Schulz, S. and Conrad, R. (1996) Influence of temperature on pathways to methane production in the permanently cold profundal sediment of Lake Constance. *FEMS Microbiol. Ecol.* 20**,** 1-14.

Schulz, S., Matsuyama, H. and Conrad, R. (1997) Temperature dependence of methane production from different precursors in a profundal sediment (Lake Constance). *FEMS Microbiol. Ecol.* 22**,** 207-213.

Schütz, H., Conrad, R., Goodwin, S. and Seiler, W. (1988) Emission of hydrogen from deep and shallow freshwater environments. *Biogeochem.* 5**,** 295-311.

Smith, J. M., Green, S. J., Kelley, C. A., Prufert-Bebout, L. and Bebout, B. M. (2008) Shifts in methanogen community structure and function associated with long-term manipulation of sulfate and salinity in a hypersaline microbial mat. *Environ. Microbiol.* 10**,** 386-394.

Smith, P. H. and Mah, R. A. (1966) Kinetics of acetate metabolism during sludge digestion. *Appl. Microbiol.* 14**,** 368-371.

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-doi: 10.1093/femsec/fiv149.

Söllinger, A., Tveit, A. T., Poulsen, M., Noel, S. J., Bengtsson, M., Bernhardt, J. et al. (2018) Holistic assessment of rumen microbiome dynamics through qunatitative metatranscriptomics reveals multifunctional redundancy during key steps of anaerobic feed degradation. *mSystems* 3**,** e00038-18-https://doi.org/10.1128/mSystems .00038-18.

Sorokin, D. Y., Abbas, B., Geleijnse, M., Pimenov, N. V., Sukhacheva, M. V. and VanLoosdrecht, M. C. M. (2015a) Methanogenesis at extremely haloalkaline conditions in the soda lakes of Kulunda Steppe (Altai, Russia). *FEMS Microbiol. Ecol.* 91**,** fiv016-doi: 10.1093/femsec/fiv016.

Sorokin, D. Y., Abbas, B., Merkel, A. Y., Rijpstra, W. I., Damste, J. S. S., Sukhacheva, M. V. and VanLoosdrecht, M. C. (2015b) *Methanosalsum natronophilum* sp.nov., and *Methanocalculus alkaliphilus* sp.nov., haloalkaliphilic methanogens from hypersaline soda lakes. *Int. J. Syst. Evol. Microbiol.* 65**,** 3739-3745.

Sorokin, D. Y., Makarova, K. S., Abbas, B., Ferrer, M., Golyshin, P. N., Galinski, E. A. et al. (2017) Discovery of extremely halophilic, methyl-reducing euryarchaea provides insights into the evolutionary origin of methanogenesis. *Nature Microbiol.* 2**,** 17081-DOI: 10.1038/nmicrobiol.2017.81.

Takai, Y. (1970) The mechanism for methane fermentation in flooded paddy soil. *Soil Sci. Plant Nutr.* 16**,** 238-244.

Thiele, J. H. and Zeikus, J. G. (1988) Control of interspecies electron flow during anaerobic digestion: significance of formate transfer versus hydrogen transfer during syntrophic methanogenesis in flocs. *Appl. Environ. Microbiol.* 54**,** 20-29.

Tyler, S. C., Rice, A. L. and Ajie, H. O. (2007) Stable isotope ratios in atmospheric CH_4: Implications for seasonal sources and sinks. *J. Geophys. Res. Atm.* 112**,** D03303-doi:10.1029/2006JD007231.

Valentine, D. L. (2007) Adaptations to energy stress dictate the ecology and evolution of the Archaea [Review]. *Nature Rev. Microbiol.* 5**,** 316-323.

VanBodegom, P. M. and Scholten, J. C. M. (2001) Microbial processes of CH4 production in a rice paddy soil: Model and experimental validation. *Geochim. Cosmochim. Acta* 65**,** 2055-2066.

VanderMaarel, M. J. E. C. and Hansen, T. A. (1997) Dimethylsulfoniopropionate in anoxic intertidal sediments: A precursor of methanogenesis via dimethyl sulfide, methanethiol, and methiolpropionate. *Mar. Geol.* 137**,** 5-12.

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*. *Nature Microbiol.* 1**,** 16170-DOI: 10.1038/NMICROBIOL.2016.170.

Vaughn, L. J. S., Conrad, M. E., Bill, M. and Torn, M. S. (2016) Isotopic insights into methane production, oxidation, and emissions in Arctic polygon tundra. *Global Change Biology* 22**,** 3487-3502.

Vavilin, V. (2012) Estimating evolution of $d^{13}CH_4$ during methanogenesis in the boreal peatland ecosystems based on stoichiometric chemical reactions, microbial dynamics and stable carbon isotope fractionation. *Ecol. Modelling* 240**,** 84-92.

Vavilin, V., Rytov, S. and Conrad, R. (2017) Modeling methane formation in sediments of tropical lakes, focusing on syntrophic acetate oxidation: dynamics and static isotope equations. *Ecol. Modeling* 363**,** 81-95.

Walter, B. P. and Heimann, M. (2000) A process-based, climate-sensitive model to derive methane emissions from natural wetlands: Application to five wetland sites, sensitivity to model parameters, and climate. *Global Biogeochem. Cycles* 14**,** 745-765.

Wang, Z. P., DeLaune, R. D., Masscheleyn, P. H. and Patrick, W. H. (1993) Soil redox and pH effects on methane production in a flooded rice soil. *Soil Sci. Soc. Am. J.* 57**,** 382-385.

Westerholm, M., Roos, S. and Schnürer, A. (2010) *Syntrophaceticus schinkii* gen. nov., sp. nov., an anaerobic, syntrophic acetate-oxidizing bacterium isolated from a mesophilic anaerobic filter. *FEMS Microbiol. Lett.* 309**,** 100-104.

Widdel, F. (1986) Growth of methanogenic bacteria in pure culture with 2-propanol and other alcohols as hydrogen donors. *Appl. Environ. Microbiol.* 51**,** 1056-1062.

Wilkins, D., Lu, X. Y., Shen, Z., Chen, J. and Lee, P. K. (2015) Pyrosequencing of *mcrA* and archaeal 16S rRNA genes reveals diversity and substrate preferences of methanogen communities in anaerobic digesters. *Appl. Environ. Microbiol.* 81**,** 604-613.

Winfrey, M. R. and Zeikus, J. G. (1979) Anaerobic metabolism of immediate methane precursors in Lake Mendota. *Appl. Environ. Microbiol.* 37**,** 244-253.

Wu, X. L., Friedrich, M. W. and Conrad, R. (2006) Diversity and ubiquity of thermophilic methanogenic archaea in temperate anoxic soils. *Environ. Microbiol.* 8**,** 394-404.

Xu, X., Elias, D. A., Graham, D. E., Phelps, T. J., Carroll, S. L., Wullschleger, S. D. and Thornton, P. E. (2015) A microbial functional group-based module for simulating methane production and consumption: Application to an incubated permafrost soil. *J. Geophys. Res. -Biogeosci.* 120**,** 1315-1333.

Yao, H., Conrad, R., Wassmann, R. and Neue, H. U. (1999) Effect of soil characteristics on sequential reduction and methane production in sixteen rice paddy soils from China, the Philippines, and Italy. *Biogeochem.* 47**,** 269-295.

Zehnder, A. J. B. (1978) Ecology of methane formation. In *Water Pollution Microbiology*. Mitchell, R. (ed). New York: Wiley, pp. 349-376.

Zeikus, J. G. (1983) Metabolic communication between biodegradative populations in nature. In *Microbes in their Natural Environments*. Slater, J. H., Whittenbury, R. and Wimpenny, J. W. T. (eds). Cambridge: Cambridge University Press, pp. 423-462.

Zhang, C., Yuan, Q. and Lu, Y. (2014) Inhibitory effects of ammonia on methanogen *mcrA* transcripts in anaerobic digester sludge. *FEMS Microbiol. Ecol.* 87**,** 368-377.

Zhang, G., Liu, G., Zhang, Y., Ma, J., Xu, H. and Yagi, K. (2013) Methanogenic pathway and fraction of CH4 oxidized in paddy fields: seasonal variation and effect of water management in winter fallow season. *Plos One* 8**,** e73982-doi:10.1371/journal.pone.0073982.

Zhuang, G. C., Elling, F. J., Nigro, L. M., Samarkin, V., Joye, S. B., Teske, A. and Hinrichs, K. U. (2016) Multiple evidence for methylotrophic methanogenesis as the dominant methanogenic pathway in hypersaline sediments from the Orca Basin, Gulf of Mexico. *Geochim. Cosmochim. Acta* 187**,** 1-20.

Zhuang, G. C., Heuer, V. B., Lazar, C. S., Goldhammer, T., Wendt, J., Samarkin, V. A. et al. (2018) Relative importance of methylotrophic methanogenesis in sediments of the Western Mediterranean Sea. *Geochim. Cosmochim. Acta* 224**,** 171-186.

Zinder, S. H. (1993) Physiological ecology of methanogens. In *Methanogenesis. Ecology, Physiology, Biochemistry and Genetics*. Ferry, J. G. (ed). New York: Chapman & Hall, pp. 128-206.

Zinder, S. H. (1994) Syntrophic acetate oxidation and "reversible acetogenesis". Drake, H. L. (ed). New York: Chapman & Hall, pp. 386-415.

Zinder, S. H. and Brock, T. D. (1978) Production of methane and carbon dioxode from methane thiol and dimethyl sulphide by anaerobic lake sediments. *Nature* 273**,** 226-228.

Figure legends

Fig. 1: Schemes of anaerobic microbial methane production (AMMP) from organic matter (e.g., cellulose) by involving the following functions (numbers in squares): (1) hydrolysis of polymeric organic matter to monomers, followed by fermentation of the monomers to short-chain fatty acids, H_2 and CO_2; (2) methanogenic conversion of acetate to CH_4 and CO_2; (3) methanogenic conversion of H_2 and CO_2 to CH_4; (4) syntrophic conversion of short-chain fatty acids to acetate, CO_2 and H_2, or of acetate to CO_2 and H_2; (5) fermentation of monomers to only acetate (heterotrophic acetogenesis) or conversion of H_2 and CO_2 to acetate (chemolithotrophic acetogenesis). (A) Complete degradation with only negligible acetogenesis (function 5); (B) Compete degradation with acetogenesis (function 5) as main fermentation process; (C) Complete degradation with aceticlastic methanogenesis (function 2) being largely replaced by syntrophic acetate oxidation (function 4); (D) incomplete degradation of organic matter; (E) Complete degradation with accumulation of acetate; (F) Complete degradation with hydrogenotrophic methanogenesis (function 3) being outcompeted by iron reduction, sulfate reduction, etc.

Fig. 2: Schemes of anaerobic microbial methane production (AMMP) from organic matter (e.g., polysaccharides) by involving the following additional functions: (A) methanogenic conversion of alcohols (function 6); (B) methanogenesis from glycolysis (function 7).

Fig. 3: Schemes of anaerobic microbial methane production (AMMP) from organic matter (e.g., pectin, xylan) involving methylotrophic methanogenesis (function 8): (A) General degradation scheme involving CH_4 production from methanol and trimethylamine (TMA); (B) methanol-dependent methanogenesis and resorption of acetate (e.g., in gut systems); (C) TMA-dependent methanogenesis and predominant utilization of acetate and H_2 by sulfate reduction.

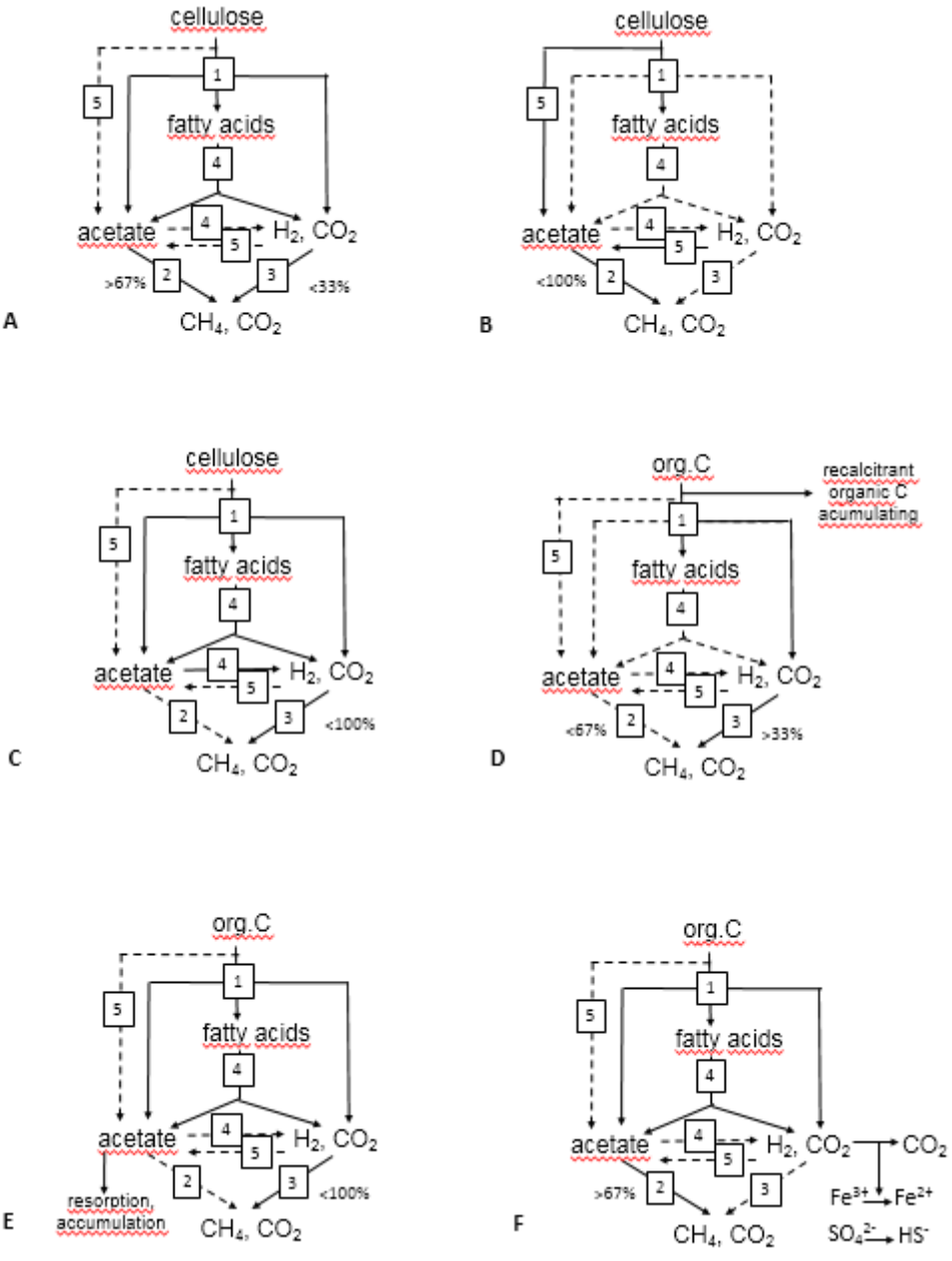

A
cellulose
fatty acids
acetate
H2, CO2
>67%
<33%
CH4, CO2
B
cellulose
fatty acids
acetate
H2, CO2
<100%
CH4, CO2
C
cellulose
fatty acids
acetate
H2, CO2
<100%
CH4, CO2
D
org.C
recalcitrant organic C accumulating
fatty acids
acetate
H2, CO2
<67%
>33%
CH4, CO2
E
org.C
fatty acids
acetate
H2, CO2
resorption, accumulation
<100%
CH4, CO2
F
org.C
fatty acids
acetate
H2, CO2
CO2
>67%
Fe3+ Fe2+
SO4 2- HS-
CH4, CO2

Fig. 1

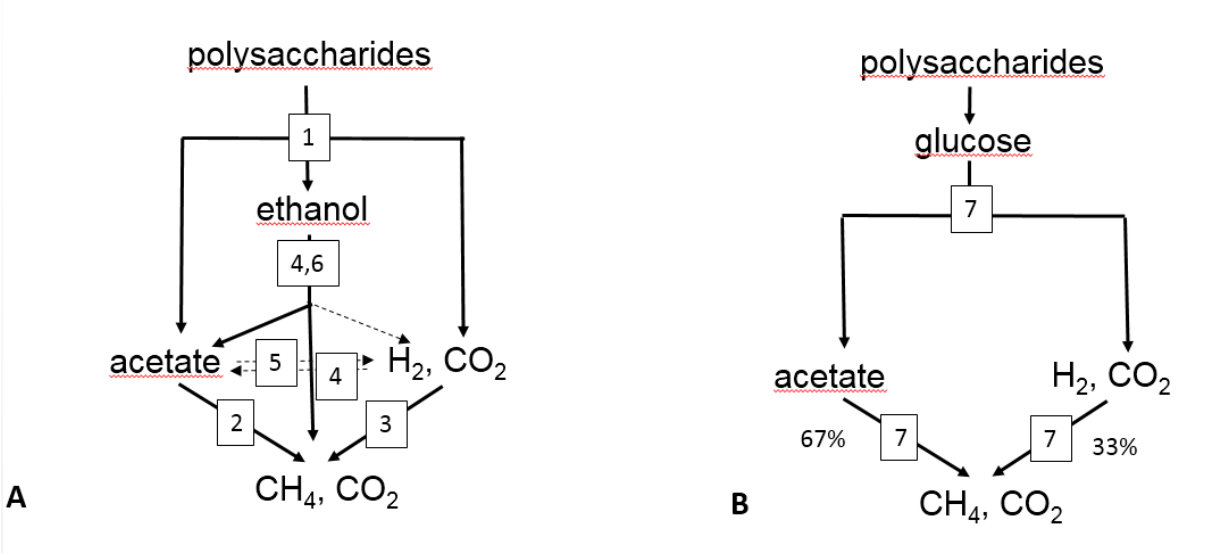
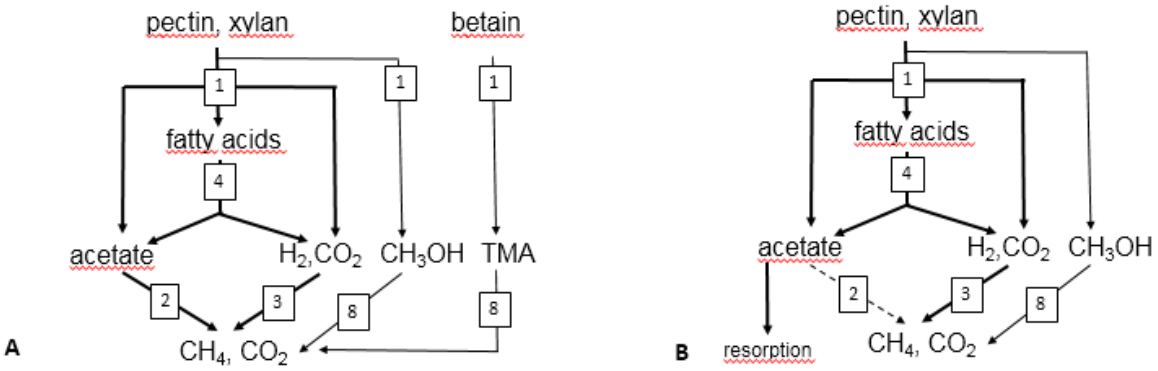

polysaccharides
1
ethanol
4,6
acetate
5
4
H_2, CO_2
2
3
CH_4, CO_2
A
polysaccharides
glucose
7
acetate
H_2, CO_2
67%
7
7
33%
CH_4, CO_2
B

Fig. 2


pectin, xylan
betain
1
1
1
fatty acids
4
acetate
H_2,CO_2
CH_3OH
TMA
2
3
8
8
CH_4, CO_2
A
pectin, xylan
1
fatty acids
4
acetate
H_2,CO_2
CH_3OH
2
3
8
resorption
CH_4, CO_2
B

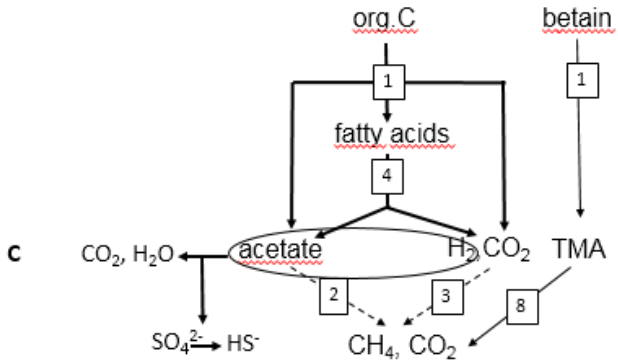

org.C
betain
1
1
fatty acids
4
CO_2, H_2O
acetate
H_2,CO_2
TMA
2
3
8
SO_4^{2-}
HS^-
CH_4, CO_2
C

Fig. 3