Methane Production in Oxic Lake Waters Potentially Increases Aquatic Methane Flux to Air

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ABSTRACT

Active methane production in oxygenated lake waters challenges the long-standing paradigm that microbial methane production occurs only in anoxic conditions, and forces us to rethink the ecology and environmental dynamics of this powerful greenhouse gas. Methane production in the upper oxic water layers places the methane source closer to the air-water interface, where convective mixing and microbubble detrainment can lead to a higher methane efflux than previously assumed. Microorganisms may produce methane in oxic environments by being equipped with enzymes to counteract the effects of molecular oxygen during methanogenesis, or using alternative pathways that do not involve oxygen-sensitive enzymes. As this process appears to be influenced by thermal stratification, water transparency and primary production, changes in lake ecology due to climate change will alter methane formation in oxic water layers, with far-reaching consequences for methane flux and climate feedback.

Introduction

As a powerful greenhouse gas, methane is projected to have 28 times the warming potential of CO_2 in the coming century.[1] Constraining the global methane budget, however, has been difficult due to uncertainties in its sources and sinks.[2,3] Methane sources can be broadly classified as biogenic, thermogenic and pyrogenic.[4] Among the biogenic sources, the prevailing paradigm is that microbial methanogenesis occurs strictly under anaerobic conditions.[5,6] Consequently, studies of methane dynamics often focus on anoxic and hypoxic habitats. This paradigm has recently been questioned due to the findings that terrestrial fungi,[7] plants[8,9] and other eukaryotes[10] can produce significant amounts of methane under oxic conditions. This novel production may substantially contribute to the total atmospheric methane and may even further increase with global warming.[11]

Bound by the prevailing paradigm, research on aquatic methane production has often ignored the upper oxic layers. For example, methane sampling in Lake Hallwil, Switzerland over the past decades had been limited to the hypolimnion, but recent measurements revealed a distinct methane peak in the oxic 7-9 m layer (McGinnis, unpublished data). A methane peak has also been recently observed in the surface waters of Lake Geneva (McGinnis, unpublished data). Likewise, decades of methane sampling in Lake Stechlin, Germany had been restricted to the sediment and bottom water, and the methane peak in the oxic metalimnion was not discovered until 2010.[12] Nevertheless, many researchers have reported inexplicable oversaturation of dissolved methane in the upper oxic waters, a phenomenon known as the “methane paradox” because methane production and accumulation are not supposed to occur in well-oxygenated waters.[2] Conventional explanations for this paradox include input from nearby anoxic sediments and shorelines,[13,14] and production within micro-anoxic zones such as detritus and animals’ gut.[15,16,17] Considering the new findings of methane formation in oxic environments on land, a revision to our fundamental understanding of the aquatic methane dynamics is needed.

Discovery of 'oxic methane production'

Keppler et al.[8] first reported that terrestrial vegetation actively releases methane under oxic conditions, and the findings were intensely debated.[18,19] Additional research further reported methane formation in oxic environments independent of methanogenic microbes.[20,21] Those studies suggest that eukaryotic methane production involves methionine[9] and other methylated precursors, and is related to environmental stressors such as reactive oxygen species.[20,21] Additionally, Angel et al.[22] showed that desert soil methanogens actively produced methane under oxic condition by overexpressing oxygen detoxifying genes. Others reported that microbes use methylated metabolites from phytoplankton to produce methane within oxic seawaters.[23-27] Collectively these findings show that methanogenesis extends beyond the traditionally perceived anoxic boundaries.

While the biochemical mechanisms behind this novel methane production remain largely unclear, the mere ability of organisms to do so forces us to re-examine the environmental dynamics of methane in aquatic ecosystems. For the purpose of this paper, we describe this as 'oxic' methane production without inferring whether or not the biochemical pathway itself requires oxygen. We review the evidence, its importance for methane flux, and the implications for microbial ecology.

Observations in aquatic systems

Tables 1 and 2 list reports of over-saturated methane concentrations in oxic sea and lake waters. While not exhaustive, the lists clearly show that the methane paradox is widespread. The reported maximum concentrations are usually much higher in freshwater (high nanomolar to micromolar levels) than in seawater (low nanomolar levels), which is consistent with the fresh-marine "dilution curve" for dissolved methane.[49] Globally, lakes cover ca. 3.7% of land[50] or 0.9% of Earth, whereas oceans cover ca. 70% of Earth. Freshwater oxic methane peaks tend to be nearly 1000-fold higher than marine oxic methane peaks, whereas the average oxic methane layer

thickness in lakes vs. oceans is ca. 1:10. Simple extrapolation suggests that the total amount of oxic freshwater methane is roughly equal to that of oxic marine methane.

One challenge in studying oxic methane production is potential interference from nearby anoxic sources. Mesocosms allow the study of the wax and wane of oxic methane production in a more controlled manner and, depending on the mesocosm design, potentially free of influences from the littoral zone and sediment. The IGB LakeLab facility in Lake Stechlin consists of 24 mesocosms (each 9 m diameter × ca. 20 m deep). Observed methane oversaturation within the oxygen-rich mesocosm water indicated that oxic methane production was independent of input from the littoral zone (Table 3), consistent with an earlier report.[47] The mesocosm bottom did not become anoxic; hence, one can rule out methane seepage from anoxic bottom as an explanation for the observations. Similar oxic methane production was observed in smaller mesocosms installed in Lac Cromwell, Canada.[46] Furthermore, all four of the monitored mesocosms developed oxic-water methane oversaturation despite their different phytoplankton compositions (based on pigments; Table 3), suggesting that oxic methane production was not dependent on a specific phytoplankton taxon.

Lake Stechlin (max. 70 m) is home to one of the longest-running limnological monitoring programs (>65 years) in north-eastern Germany. Methane production in the upper oxic layer has been repeatedly observed since 2010,[12,47] coinciding with the phytoplankton growth season, and methane concentration within the upper 25 m was linearly correlated with primary production.[47] Positive correlations between oxic-water methane and chlorophyll concentrations in several seas and lakes have also been reported.[46,51] Together, these observations suggest that the oxic methane production is associated with primary production.

Methane can be rapidly oxidized by methanotrophs to CO_2 in the presence of oxygen, as often seen in the water layer overlying anoxic sediment. Using molecular markers, Grossart et al.[12] detected the presence of methane oxidizers only below the thermocline but not within the oxic methane peak in Lake Stechlin. Murase and

Sugimoto[52] incubated Lake Biwa waters under different light intensities and reported lower oxidation rates in the light. Similar photoinhibition effects were also found in Lake Stechlin[47] (Table 4). The absence or photoinhibition of methane oxidizers thereby allows for the accumulation of methane in the upper oxic water column.

Implications for lake-to-air methane flux

Diffusive methane flux F_i from water to the atmosphere is determined by the methane concentration at the surface water $C_{w,}$ the atmospheric saturation concentration C_{sat} (~3 nM) and the physical processes driving the water-air exchange coefficient k (m d^{-1}):[53]

$$F_i = k\,(C_w - C_{sat})$$

In the case of anoxic bottom methane production in deep stratified lakes, the thermocline acts as a barrier that 1) physically limits the upward flux from bottom water, and 2) allows methanotrophs to oxidize methane within the oxic zone subsequently fortifying that barrier. Hence, significant exposure of dissolved methane to the atmosphere is limited to periods of deep convective mixing or complete lake turnover. However, even in the latter case there is still uncertainty as to how much methane will reach the atmosphere and how much is oxidized.[54,55]

With the methane source located in the upper oxic layer instead of the bottom (Fig. 1), methane only needs to be transported over a much shorter distance to reach the water-air interface. Additionally, shallow water mixing (convection), which often occurs diurnally, both exposes higher methane concentrations to the air-water interface and enhances k.[53] These fluxes would be particularly important during periods of colder weather and higher winds during the stratified season, and would be further elevated by microbubbles.[54] These additional mechanisms for releasing methane from the surface are not considered in conventional Fickian diffusion (k) calculations.[54,56]

Bastviken et al.[57] estimates that freshwaters contribute 103.3 Tg CH_4 yr^{-1} to the atmosphere. Of this, they attribute 9.5% to diffusive fluxes with an average of 0.51 mmol m^{-2} d^{-1} covering arctic to tropical lakes (n = 397). The data, however, rarely included night-time measurements when convection was strongest, and had very limited seasonal studies. Most diffusive fluxes for their budget estimate relied on parameterizations for *k* based on wind speed,[58,59] which tend to underestimate surface diffusive fluxes, particularly during convective mixing due to surface cooling that strongly drives *k* values[53] or microbubble flux enhancement.[54] Convection-driven *k* can increase the flux as much as five times over the wind parameterization.[60]

The near-surface oxic methane sources combined with more realistic estimates for transport will increase the estimated contribution to the global budget. As an illustrative example, fluxes from Lake Stechlin ranged from 0.95 mmol m^{-2} d^{-1} in July 2014 (avg. surface CH_4 0.28 µmol L^{-1}, wind speed 1.9 m s^{-1}, our unpublished data) to 2.7 mmol m^{-2} d^{-1} in August 2013 (avg. surface CH_4 0.37 µmol L^{-1}, wind speed 4.2 m s^{-1}).[54] These values are 1.9-5.3 times higher than Bastviken et al.'s estimates, suggesting that diffusive emissions from lakes, particularly due to the oxic methane peak, could be doubled (~18.5 Tg yr^{-1}) or even higher.

Implications for aquatic microbial ecology

How microbes produce methane under oxic condition is unclear. We consider two possibilities: 1) They use conventional biochemical pathways but are also equipped with ways to counteract the effects of oxygen; 2) They use biochemical pathways that do not involve oxygen-sensitive enzymes as described for the conventional pathways.

In the conventional pathways, the carbon-borne precursor molecules act as electron acceptors in a series of redox reactions releasing methane as the end product. Although this process is supposedly wide-spread in the oxygen-free ancient ocean, it is wasteful because the energy-rich methane is lost. With the advent of oxygenic photosynthesis, oxygen becomes the preferred electron acceptor as more energy can

be generated. This 'switch' from a fully anaerobic metabolism to an exclusively aerobic metabolism requires major changes in the cell's genetic blueprint and biochemical machinery, and leads to an evolutionary divergence of aerobes from their anaerobic ancestors.[6] Anaerobic organisms became marginalized over time to the remaining anoxic fringe habitats in lakes and oceans. However, some ancestral anaerobes, without committing themselves to whole-sale changes, may have developed ways to neutralize the negative effects of oxygen and continue to occupy the vast but increasingly oxygenated environment. Many oxygen-tolerant microorganisms have the antioxidant enzyme catalase, which can be coded for by a single gene.[61,62] This strategy appears to be employed by desert soil methanogens.[22]

Alternatively, microbes may use pathways not affected by oxygen (Fig. 2). Karl et al.[23] suggest that microbes in the equatorial Pacific break down methylphosphonate (MPn) and release methane as a by-product. The process requires enzymatic cleavage of the C-P bond and is not oxygen sensitive. The operon for the C-P lyase enzyme PhnJ responsible for breaking the C-P bond[63] is widespread across the bacterial domain.[64] Accordingly, this reaction can be catalysed by numerous phosphorus scavenging microorganisms, and supports the notion that MPn is a main phosphorus source for microbes in oligotrophic waters.[65,66] While biological phosphonate production is common within the bacterial domain,[67] currently only one methylphosphonate synthase (mpnS) has been identified originating from the marine *Thaumarchaeota*.[68] These organisms are abundant in the ocean, although they have been reported in some freshwater lakes as well.[69] Another probable source in freshwater are the *Actinobacteria*, which produce a large diversity of phosphonate compounds.[70,71] The high abundance of freshwater *Actinobacteria* coupled with known C-P lyase activity of cyanobacteria[72] may explain the correlation between oxic methane formation and cyanobacteria bloom in Lake Stechlin.[12] Damm et al.[25] suggest that arctic microbes metabolize dimethylsulfoniopropionate (DMSP) (requiring enzymatic cleavage of the C-S bond) for energy production and release methane as a by-product, which would require a final step of methyl reduction. However, the methyl reductase Mcr gene complex has not been found in any non-methanogenic genome and has no known

structural homolog in *Bacteria.* To allow the process to occur in oxic water, Damm et al.[73] theorize that DMSP-utilizing bacteria maintain an anoxic cytoplasm through respiration, although empirical evidence is still missing.

From an energetic standpoint it is hardly favourable to discard methane as a by-product; nevertheless, the implication of the earlier work is that oxic methane production could be driven by microbes equipped with C-P lyase or C-S lyase,[23,25] which are common among heterotrophic microbes capable of metabolizing C-1 compounds.[74] A comparative genomics analysis shows that the majority of enzymes in the various methanogenic pathways are present in non-methanogenic organisms including *Bacteria* (Fig. 2). This along with the presence of several C1 carriers (tetrahydrofolate and tetrahydromethanoptrin)[75] among *Bacteria* allows us to speculate that upon demethylation of C-1 compounds, the methyl group bound to a C1-carrier or an unknown Coenzyme-M homolog is reduced to methane by cellular reductases, for which the methyl-reductase function has not been identified (Fig. 2). Alternative sources of reducing power potentially include: 1) Electron bifurcation that has been described for anaerobic methanogenesis[76] but not yet for oxic methane production; 2) reducing power dumping by photosystems (in cyanobacteria) or proteorhodopsin (in *Bacteria*), especially under nutrient limitation.

There is emerging evidence that some microalgal species may directly produce methane by demethylation, completely by-passing the involvement of heterotrophic microbes.[77] Organosulfur compounds such as methionine, dimethyl sulfoxide and DMSP are commonly produced by algae. It has been reported that, under ambient atmospheric condition, several organosulfur compounds can be chemically converted to methane.[9] If similar processes are confirmed in algae, methane production in oxic waters would be much more pervasive than previously imagined.

Implications for climate and future research directions

Cyanobacteria blooms are on the rise due to eutrophication and climate change.[78] Given that strong oxic methane production has been associated with cyanobacteria blooms,[12] this could result in a positive greenhouse feedback. Meanwhile, the fate of the oxic methane source is influenced by stratification pattern and surface mixing events, but these processes may not be fully captured by climate models, especially for small lakes.[79]

Methane has long been the focus in ecological and climate research, but the current view of its global dynamics is biased by the conventional exclusion of oxic habitats and processes.[3,4] In light of the new findings discussed here, it is necessary to revisit the century-old understanding of aquatic microbial methane production and address several urgent research areas: 1) More research is needed on the precise biochemical pathway(s) behind oxic methane production, and the use of stable isotopes and tracers can shed light into the different precursor compounds and pathways; 2) Further investigation is warranted on the fate of this novel methane source, including water-to-air exchange and internal consumption via methanotrophy; 3) Isolation and cultivation of the responsible organisms will be needed for detailed physiological studies; 4) It is necessary to revisit the global methane budget by including oxic methane sources, and the role they may play in future climate.

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500 Table 1. Some examples of studies reporting over-saturated methane concentrations in oxic seawaters.

Location	Observations	Reference
Western subtropical N. Atlantic	• CH_4 oversaturation in the upper 300 m • Max. 3.5 nM CH_4 overlapping thermocline • Physical transport could not explain observed CH_4 peak	Scranton and Brewer[28]
North Atlantic 35°S to 50°N	• CH_4 oversaturation in the upper 1000 m; max. ~4 nM CH_4 • CH_4 conc. not correlated with chlorophyll or hydrogen	Conrad and Seiler[29]
Southern California Bight, U.S.	• CH_4 peak (ca. 8 nM) overlapping thermocline and oxycline • Almost no CH_4 oxidation in the upper 100 m	Ward and Kilpatrick[30]
Western North Pacific	• High CH_4 (2.2-3.4 nM) within 0-200 m • CH_4 poorly correlated with chlorophyll in upper 100 m	Watanabe et al.[31]
California coast, U.S.; VERTEX stations	• Max. 5.42 nM CH_4 in upper 200 m • Zooplankton guts and sinking particles were suggested as the source	Tilbrook and Karl[32]
Arabian Sea	• Weakly developed CH_4 max. in the upper 50 m • More pronounced CH_4 max. (up to 8.5 nM) at 150-200 m coinciding max. NO_2 and beam attenuation	Jayakumar et al.[33]
Monterey Bay, U.S.	• CH_4 accumulated at thermocline (100-200 m) • CH_4 bubbles from deep water were unlikely the source	Rehder et al.[34] McGinnis et al.[35]
Western subarctic gyre of N. Pacific	• Up to 12% CH_4 oversaturation in the upper 100 m • Sinking particles were suggested as the source	Sasakawa et al.[36]
Fram Strait	• High CH_4 (7-9 nM) overlapping high O_2 (380-390 μmol l^{-1}) in upper 20 m	Damm et al.[37]
Japan Sea	• Average 2.6 nM CH_4 at surface; max. 14 nM at ~50 m • Sediment CH_4 was unlikely the source	Vereshchagina et al.[38]
Central Chile upwelling system	• 125-550% CH_4 saturation at 0-30 m with >100% O_2 • CH_4 oversaturation coincided with seasonal upwelling, high chlorophyll and high DMSP levels.	Florez-Leiva et al.[39]
ALOHA station	• CH_4 oversaturation down to 175 m • Max. ~3.6 nM CH_4 coincided with max. 226 μM O_2	Del Valle and Karl[24]

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504 Table 2. Some examples of studies reporting over-saturated methane concentrations in oxic lake waters.

Location	Observations	Reference
Lake 227, Canada	• CH_4 decreased from 282.5 µM at 9 m to ~0.5 µM at 7.5 m • Both CH_4 and oxidation remained low in surface layer	Rudd et al.[40]
Lake St. George, Canada	• CH_4 peak (~5 µM) at 6 m overlapping thermocline, NH_4 and NO_3 peaks; oxidation activity not detectable	Bedard and Knowles[41]
Lake Biwa, Japan	• Station A: max. ~175 nM CH_4 coinciding with thermocline and ~250 µmol O_2 l^{-1} • Station B: max. 205 nM CH_4 coinciding with thermocline and ~188 µmol O_2 l^{-1} • River runoff, littoral and sublittoral sediments were suggested as the source	Murase et al.[13]
Lakes in south central Sweden	• High CH_4 (~140 µM) near sediment (11 m) • No upper CH_4 peak was observed • High CH_4 oxidation activity below 5 m.	Sundh et al.[42]
Lake Paul and Lake Peter, USA	• CH_4 was nearly zero at thermocline and oxygen peak at 5 m, then increased to 4 µM in the surface layer	Bastviken et al.[43]
10 boreal lakes in Finland	• High CH_4 (> 1 µM) in surface layer with 60 to >100% O_2 saturation in some of the lakes	Juutinen et al.[44]
Sakinow Lake, Canada	• CH_4 was low (0.02-0.1 µM) at thermocline depth (~20 m), then increased to ~0.35 µM at the surface • CH_4 bubbles from sediment could not explain high CH_4 in surface water	Vagle et al.[45]
Lake Constance, Germany	• High CH_4 (1.5 µM) above thermocline • Lateral transport from littoral zone was suggested as the source	Hofmann et al.[14]
Lake Stechlin, Germany	• Low CH_4 (<0.2 µM) in hypolimnion • Max. ~1.4 µM CH_4 in metalimnion overlapping oxygen peak • Methanotrophs absent in metalimnion • Experiments confirmed active CH_4 production in oxic water	Grossart et al.[12]
Lac Cromwell, Canada	• High CH_4 (0.10-0.53 µM) in mesocosms under oxic condition (45.6-128.6% O_2 saturation)	Bogard et al.[46]
Nine lakes in NE Germany	• CH_4 positively correlated with O_2 in surface waters • Euphotic zone CH_4 positively correlated with primary production • Ebullition from sediment was unlikely the source	Tang et al.[47]
Lake Lugano, Switzerland	• High CH_4 (up to 180 nM) in the upper oxic layer in stratification season • Vertical profiles suggest excess CH_4 from a near-surface source	Blees et al.[48]

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Table 3. Methane measurements in four mesocosms within the LakeLab in September, 2012. The mes
20 m deep with a thermocline at ca. 8 m, and were dominated by different phytoplankton based on pig
methane concentration, maximum and minimum methane concentrations and the corresponding disso
depths are shown. Pigments were measured by BBE (Kiel) probe; temperature and oxygen were meas
(Weilheim) submersible probe; CH_4 of discrete depth water samples was measured by standard heads
method.[12,47]

Dominant phytoplankton	Surface CH_4 (µM)	Max. CH_4 (µM)	D.O. (mg l^{-1})	Depth (m)	Min. CH_4 (µM)	D.O. (mg l^{-1})
Green algae	0.11	0.11	11.6	3	0.05	8.7
Chryptophyte and cyanobacteria	0.11	0.12	11.6	3	0.03	8.7
Green algae and diatoms	0.10	0.12	6.9	15	0.06	8.0
Green algae	0.23	0.23	9.13	0	0.13	8.3

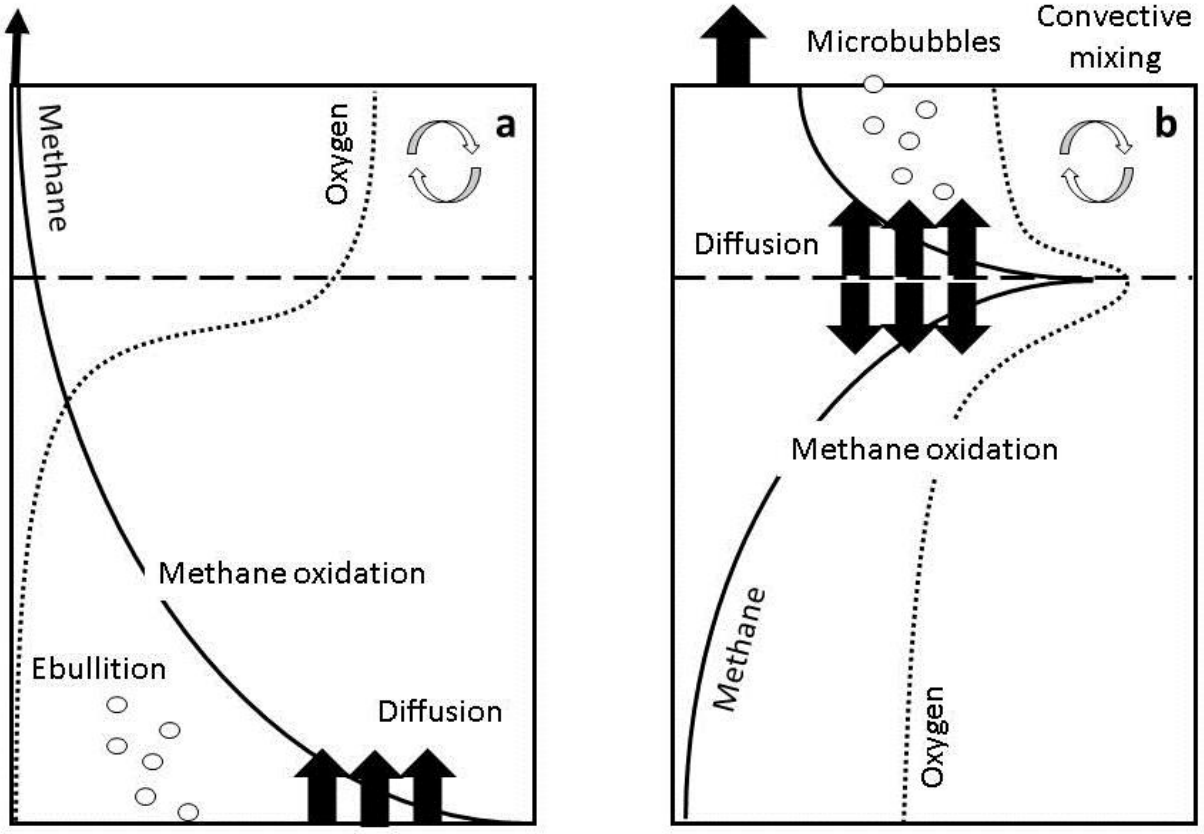
Table 4. Methane oxidation rates under light and dark conditions. Values for Lake Biwa are calculated for the first 3 days from Fig. 2B and 2C of Murase and Sugimoto.[52]

Location	Methane oxidation rate (nmol L^{-1} d^{-1})		Reference
	Light	Dark	
Lake Biwa (thermocline)	0.33	2.67	Murase and Sugimoto[52]
Lake Biwa (hypolimnion)	26	55	
Lake Stechlin	89	103	Tang et al.[47]

Figure Legend

Fig. 1. Comparison of two scenarios of methane dynamics in a stratified water column; a: The traditional scenario where methane is produced in the anoxic sediments, transported upward by diffusion and ebullition, and is rapidly consumed by methane oxidation in the hypolimnion, resulting in no or little methane outflux from the system; b: The alternative scenario where oxic methane production occurs in the surface layer. Convective mixing, microbubble detrainment and diffusion in the epilimnion result in higher methane outflux from the system. Downward diffusion also fuels methane oxidation in the hypolimnion. Thermocline is indicated by the dashed line.

Fig. 2. Known and hypothetical methanogenesis pathways. EC numbers for catalysing enzymes marked in green are found in genome annotation of non-methanogenic organisms including *Bacteria* (i.e. non Archaea; based on KEGG taxonomy and PATRIC). Precursor compounds reported for oxic methane production are marked in blue (authors' unpubl. data marked by ∗). Pathways known to require anoxic environments are grouped in black frames. Known and hypothesized pathways that occur in oxic conditions are grouped within solid or dashed red frames, respectively. See text for details.



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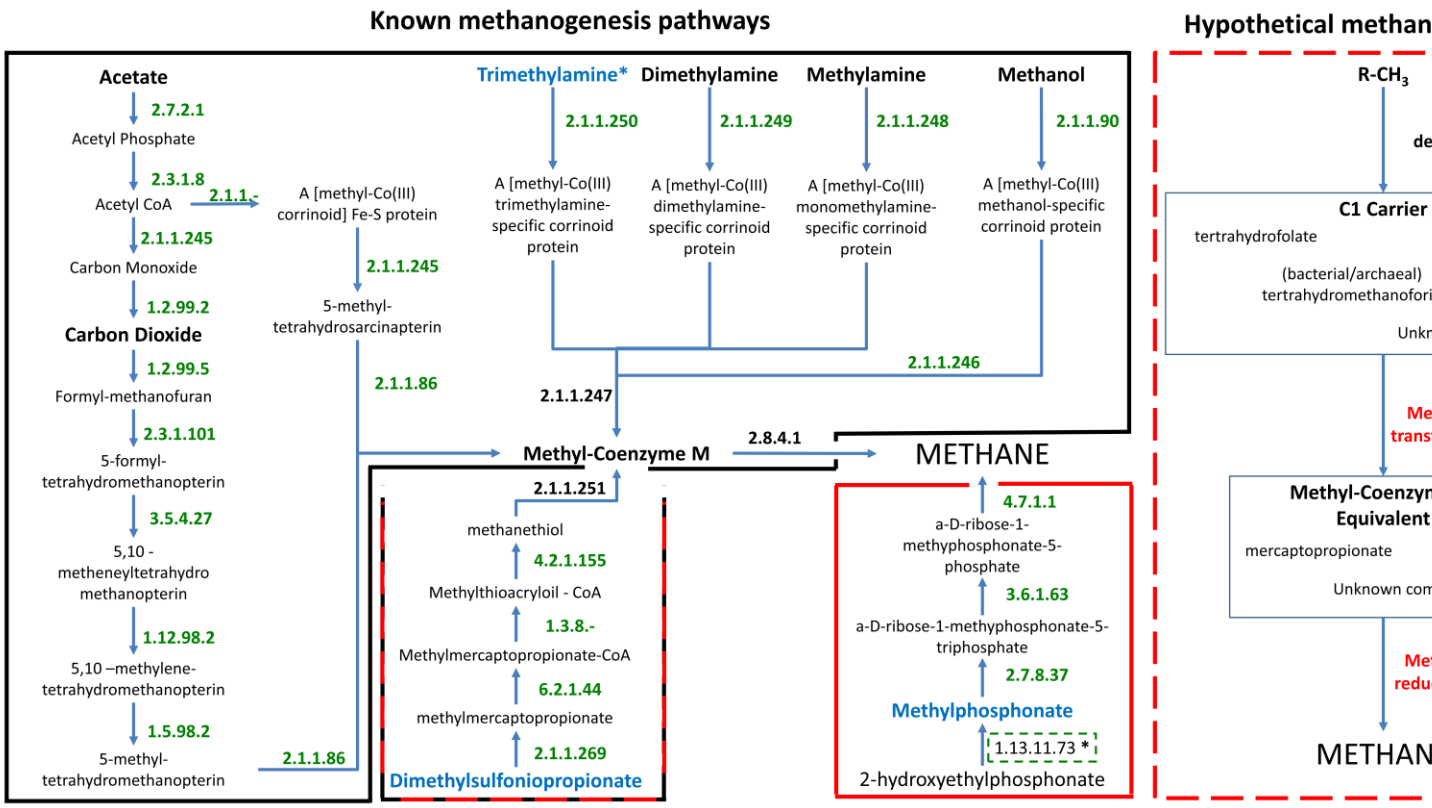

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Kam W. Tang, Daniel F. McGinnis, Danny Ionescu, Hans-Peter Grossart