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### **Paper:**

Tang, K., McGinnis, D., Ionescu, D. & Grossart, H. (2016). Methane Production in Oxic Lake Waters Potentially Increases Aquatic Methane Flux to Air. *Environmental Science & Technology Letters*, 3(6), 227-233.  
<http://dx.doi.org/10.1021/acs.estlett.6b00150>

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

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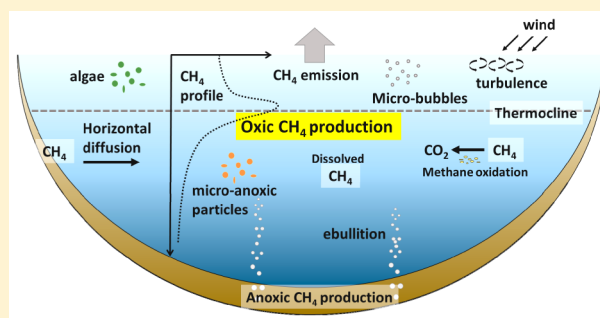
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**ABSTRACT:** Active methane production in oxygenated lake waters challenges the long-standing paradigm that microbial methane production occurs only under anoxic conditions and forces us to rethink the ecology and environmental dynamics of this powerful 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 methane efflux higher than that 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<sub>2</sub> in the coming century.<sup>1</sup> Constraining the global methane budget, however, has been difficult because of uncertainties in its sources and sinks.<sup>2,3</sup> Methane sources can be broadly classified as biogenic, thermogenic, and pyrogenic.<sup>4</sup> Among the biogenic sources, the prevailing paradigm is that microbial methanogenesis occurs strictly under anaerobic conditions.<sup>5,6</sup> Consequently, studies of methane dynamics often focus on anoxic and hypoxic habitats. This paradigm has recently been questioned because of the findings that terrestrial fungi,<sup>7</sup> plants,<sup>8,9</sup> and other eukaryotes<sup>10</sup> 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.<sup>11</sup>

Bound by the prevailing paradigm, research on aquatic methane production has often ignored the upper oxic layers. For example, methane sampling in Lake Hallwil in 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 (D. F. McGinnis, unpublished data). A methane peak has also been recently observed in the surface waters of Lake Geneva (D. F. McGinnis, unpublished data). Likewise, decades of methane sampling in Lake Stechlin in Germany had been restricted to the sediment and bottom water, and the methane peak in the oxic metalimnion was not discovered until 2010.<sup>12</sup> 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.<sup>2</sup> Conventional explanations for this paradox include input from nearby anoxic sediments and shorelines<sup>13,14</sup> and production within microanoxic zones such as detritus and animals’ gut.<sup>15–17</sup> 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”

Keppeler et al.<sup>8</sup> first reported that terrestrial vegetation actively releases methane under oxic conditions, and the findings were intensely debated.<sup>18,19</sup> Additional research further reported methane formation in oxic environments in a manner independent of methanogenic microbes.<sup>20,21</sup> Those studies suggest that eukaryotic methane production involves methionine<sup>9</sup> and other methylated precursors and is related to environmental stressors such as reactive oxygen species.<sup>20,21</sup> Additionally, Angel et al.<sup>22</sup> showed that desert soil methanogens actively produced methane under oxic condition by overexpressing oxygen-detoxifying genes. Others reported that

Received: May 4, 2016

Revised: May 30, 2016

Accepted: May 31, 2016

Published: May 31, 2016

**Table 1. Some Examples of Studies Reporting Oversaturated Methane Concentrations in Oxic Seawaters**

location	observations	ref
western subtropical North Atlantic	CH <sub>4</sub> oversaturation in the upper 300 m; maximum of 3.5 nM CH <sub>4</sub> overlapping thermocline; physical transport could not explain observed CH <sub>4</sub> peak	Scranton and Brewer <sup>28</sup>
North Atlantic from 35°S to 50°N	CH <sub>4</sub> oversaturation in the upper 1000 m; maximum of ~4 nM CH <sub>4</sub> ; CH <sub>4</sub> concentration not correlated with chlorophyll or hydrogen	Conrad and Seiler <sup>29</sup>
Southern California Bight, United States	CH <sub>4</sub> peak (~8 nM) overlapping thermocline and oxycline; almost no CH <sub>4</sub> oxidation in the upper 100 m	Ward and Kilpatrick <sup>30</sup>
western North Pacific	high CH <sub>4</sub> (2.2–3.4 nM) within 0–200 m; CH <sub>4</sub> poorly correlated with chlorophyll in upper 100 m	Watanabe et al. <sup>31</sup>
California coast, United States; VERTEX stations	maximum of 5.42 nM CH <sub>4</sub> in upper 200 m; zooplankton guts and sinking particles were suggested as the source	Tilbrook and Karl <sup>32</sup>
Arabian Sea	weakly developed CH <sub>4</sub> maximum in the upper 50 m; more pronounced CH <sub>4</sub> maximum (up to 8.5 nM) at 150–200 m coinciding with maximal NO <sub>2</sub> and beam attenuation	Jayakumar et al. <sup>33</sup>
Monterey Bay, United States	CH <sub>4</sub> accumulated at thermocline (100–200 m); CH <sub>4</sub> bubbles from deep water were unlikely the source	Rehder et al., <sup>34</sup> McGinnis et al. <sup>35</sup>
western subarctic gyre of North Pacific	up to 12% CH <sub>4</sub> oversaturation in the upper 100 m; sinking particles were suggested as the source	Sasakawa et al. <sup>36</sup>
Fram Strait	high CH <sub>4</sub> (7–9 nM) overlapping high O <sub>2</sub> (380–390 μmol L <sup>-1</sup> ) in upper 20 m	Damm et al. <sup>37</sup>
Japan Sea	average 2.6 nM CH <sub>4</sub> at surface; maximum of 14 nM at ~50 m; sediment CH <sub>4</sub> was unlikely the source	Vereshchagina et al. <sup>38</sup>
central Chile upwelling system	125–550% CH <sub>4</sub> saturation at 0–30 m with >100% O <sub>2</sub> ; CH <sub>4</sub> oversaturation coincided with seasonal upwelling, high chlorophyll levels, and high DMSP levels	Florez-Leiva et al. <sup>39</sup>
ALOHA station	CH <sub>4</sub> oversaturation down to 175 m; maximum of ~3.6 nM CH <sub>4</sub> coincided with maximum of 226 μM O <sub>2</sub>	Del Valle and Karl <sup>24</sup>

**Table 2. Some Examples of Studies Reporting Oversaturated Methane Concentrations in Oxic Lake Waters**

location	observations	ref
Lake 227, Canada	CH <sub>4</sub> decreased from 282.5 μM at 9 m to ~0.5 μM at 7.5 m; both CH <sub>4</sub> and oxidation remained low in surface layer	Rudd et al. <sup>40</sup>
Lake St. George, Canada	CH <sub>4</sub> peak (~5 μM) at 6 m overlapping thermocline, NH <sub>4</sub> , and NO <sub>3</sub> peaks; oxidation activity not detectable	Bedard and Knowles <sup>41</sup>
Lake Biwa, Japan	station A, maximum of ~175 nM CH <sub>4</sub> coinciding with thermocline and ~250 μmol of O <sub>2</sub> L <sup>-1</sup> ; station B, maximum of 205 nM CH <sub>4</sub> coinciding with thermocline and ~188 μmol of O <sub>2</sub> L <sup>-1</sup> ; river runoff, littoral and sublittoral sediments were suggested as the source	Murase et al. <sup>13</sup>
Lakes in south central Sweden	high CH <sub>4</sub> (~140 μM) near sediment (11 m); no upper CH <sub>4</sub> peak was observed; high CH <sub>4</sub> oxidation activity below 5 m	Sundh et al. <sup>42</sup>
Lake Paul and Lake Peter, United States	CH <sub>4</sub> was nearly zero at thermocline and oxygen peak at 5 m and then increased to 4 μM in the surface layer	Bastviken et al. <sup>43</sup>
10 boreal lakes in Finland	high CH <sub>4</sub> (>1 μM) in surface layer with 60 to >100% O <sub>2</sub> saturation in some of the lakes	Juutinen et al. <sup>44</sup>
Sakinow Lake, Canada	CH <sub>4</sub> was low (0.02–0.1 μM) at thermocline depth (~20 m) and then increased to ~0.35 μM at the surface; CH <sub>4</sub> bubbles from sediment could not explain high CH <sub>4</sub> in surface water	Vagle et al. <sup>45</sup>
Lake Constance, Germany	high CH <sub>4</sub> (1.5 μM) above thermocline; lateral transport from littoral zone was suggested as the source	Hofmann et al. <sup>14</sup>
Lake Stechlin, Germany	low CH <sub>4</sub> (<0.2 μM) in hypolimnion; maximum of ~1.4 μM CH <sub>4</sub> in metalimnion overlapping oxygen peak; methanotrophs absent in metalimnion; experiments confirmed active CH <sub>4</sub> production in oxic water	Grossart et al. <sup>12</sup>
Lac Cromwell, Canada	high CH <sub>4</sub> (0.10–0.53 μM) in mesocosms under oxic conditions (45.6–128.6% O <sub>2</sub> saturation)	Bogard et al. <sup>46</sup>
nine lakes in northeast Germany	CH <sub>4</sub> positively correlated with O <sub>2</sub> in surface waters; euphotic zone CH <sub>4</sub> positively correlated with primary production; ebullition from sediment was unlikely the source	Tang et al. <sup>47</sup>
Lake Lugano, Switzerland	high CH <sub>4</sub> (up to 180 nM) in the upper oxic layer in stratification season; vertical profiles suggest excess CH <sub>4</sub> from a near-surface source	Blees et al. <sup>48</sup>

microbes use methylated metabolites from phytoplankton to produce methane within oxic seawaters.<sup>23–27</sup> 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 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 oversaturated methane concentrations in oxic sea and lake waters. While not exhaustive, the lists clearly show that the methane paradox is widespread. The reported maximal 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.<sup>49</sup> Globally, lakes cover ~3.7% of land<sup>50</sup> or 0.9% of Earth, whereas oceans cover ~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 ratio in lakes to oceans is ~1:10. Simple extrapolation

**Table 3. Methane Measurements in Four Mesocosms within the LakeLab in September 2012<sup>a</sup>**

dominant phytoplankton	surface CH <sub>4</sub> (μM)	maximal CH <sub>4</sub> (μM)	DO (mg L <sup>-1</sup> )	depth (m)	minimal CH <sub>4</sub> (μM)	DO (mg L <sup>-1</sup> )	depth (m)
green algae	0.11	0.11	11.6	3	0.05	8.7	13
chryptophyte and cyanobacteria	0.11	0.12	11.6	3	0.03	8.7	13
green algae and diatoms	0.10	0.12	6.9	15	0.06	8.0	12
green algae	0.23	0.23	9.13	0	0.13	8.3	6

<sup>a</sup>The mesocosms were ~20 m deep with a thermocline at ~8 m and were dominated by different phytoplankton based on pigment data. Surface methane concentration, maximal and minimal methane concentrations, and the corresponding dissolved oxygen (DO) and depths are listed. Pigments were measured by BBE (Kiel) probe. The temperature and oxygen level were measured by a WTW (Weilheim) submersible probe. CH<sub>4</sub> of discrete depth water samples was measured by the standard headspace displacement method.<sup>12,47</sup>

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 in diameter × ~20 m in depth). 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.<sup>47</sup> The mesocosm bottom did not become anoxic; hence, one can rule out seepage of methane from the anoxic bottom as an explanation for the observations. Similar oxic methane production was observed in smaller mesocosms installed in Lac Cromwell in Canada.<sup>46</sup> 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 (maximum of 70 m) is home to one of the longest-running limnological monitoring programs (>65 years) in northeastern Germany. Methane production in the upper oxic layer has been repeatedly observed since 2010,<sup>12,47</sup> coinciding with the phytoplankton growth season, and the methane concentration within the upper 25 m was linearly correlated with primary production.<sup>47</sup> Positive correlations between oxic–water–methane and chlorophyll concentrations in several seas and lakes have also been reported.<sup>46,51</sup> Together, these observations suggest that oxic methane production is associated with primary production.

Methane can be rapidly oxidized by methanotrophs to CO<sub>2</sub> in the presence of oxygen, as often seen in the water layer overlaying the anoxic sediment. Using molecular markers, Grossart et al.<sup>12</sup> detected the presence of methane oxidizers only below the thermocline but not within the oxic methane peak in Lake Stechlin. Murase and Sugimoto<sup>52</sup> 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<sup>47</sup> (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 surface water  $C_w$ , atmospheric saturation concentration  $C_{sat}$  (~3 nM), and the physical processes driving water–air exchange coefficient  $k$  (meters per day).<sup>53</sup>

**Table 4. Methane Oxidation Rates under Light and Dark Conditions<sup>a</sup>**

location	methane oxidation rate (nmol L <sup>-1</sup> day <sup>-1</sup> )		ref
	light	dark	
Lake Biwa (thermocline)	0.33	2.67	Murase and Sugimoto <sup>52</sup>
Lake Biwa (hypolimnion)	26	55	
Lake Stechlin	89	103	Tang et al. <sup>47</sup>

<sup>a</sup>Values for Lake Biwa are calculated for the first 3 days from panels B and C of Figure 2 of ref 52.

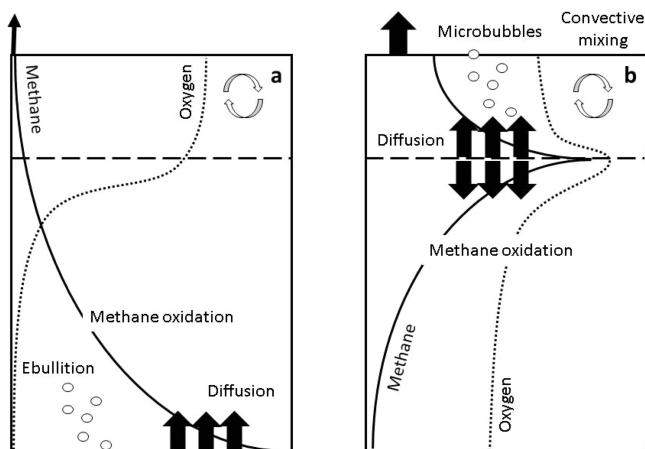
$$F_i = k(C_w - C_{sat})$$

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 about how much methane will reach the atmosphere and how much is oxidized.<sup>54,55</sup>

With the methane source located in the upper oxic layer instead of the bottom (Figure 1), methane needs to be transported over only 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  $k$ .<sup>53</sup> 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.<sup>54</sup> These additional mechanisms for releasing methane from the surface are not considered in conventional Fickian diffusion ( $k$ ) calculations.<sup>54,56</sup>

Bastviken et al.<sup>57</sup> estimated that freshwaters contribute 103.3 Tg of CH<sub>4</sub> year<sup>-1</sup> to the atmosphere. Of this, they attribute 9.5% to diffusive fluxes with an average of 0.51 mmol m<sup>-2</sup> day<sup>-1</sup> covering Arctic to tropical lakes ( $n = 397$ ). The data, however, rarely included night-time measurements when convection was strongest, and there has been very limited seasonal study. Most diffusive fluxes for their budget estimate relied on parametrizations for  $k$  based on wind speed,<sup>58,59</sup> which tend to underestimate surface diffusive fluxes, particularly during convective mixing due to surface cooling that strongly drives  $k$  values<sup>53</sup> or microbubble flux enhancement.<sup>54</sup> Convection-driven  $k$  can increase the flux as much as 5 times over the wind parametrization.<sup>60</sup>

The near-surface oxic methane sources combined with more realistic estimates for transport will increase the estimated



**Figure 1.** Comparison of two scenarios of methane dynamics in a stratified water column. (a) Traditional scenario in which methane is produced in the anoxic sediments, transported upward by diffusion and ebullition, and rapidly consumed by methanogenesis in the hypolimnion, resulting in no or little methane outflux from the system. (b) Alternative scenario in which 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 methanogenesis in the hypolimnion. Thermocline is indicated by the dashed line.

contribution to the global budget. As an illustrative example, fluxes from Lake Stechlin ranged from 0.95 mmol m<sup>-2</sup> day<sup>-1</sup> in July 2014 [average surface CH<sub>4</sub> of 0.28 μmol L<sup>-1</sup>, wind speed of 1.9 m s<sup>-1</sup> (our unpublished data)] to 2.7 mmol m<sup>-2</sup> day<sup>-1</sup> in August 2013 (average surface CH<sub>4</sub> of 0.37 μmol L<sup>-1</sup>, wind speed of 4.2 m s<sup>-1</sup>).<sup>54</sup> These values are 1.9–5.3 times higher than the estimates of Bastviken et al., suggesting that diffusive

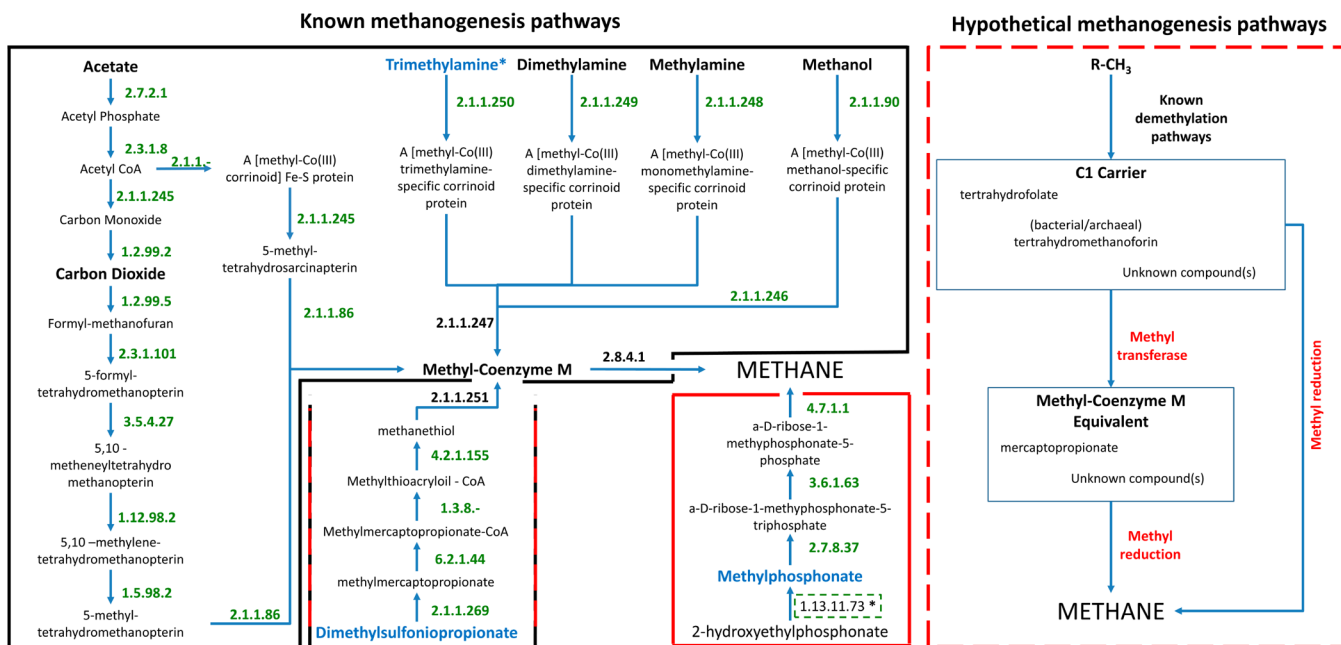
emissions from lakes, particularly due to the oxic methane peak, could be doubled (~18.5 Tg year<sup>-1</sup>) or even higher.

**■ IMPLICATIONS FOR AQUATIC MICROBIAL ECOLOGY**

How microbes produce methane under oxic conditions 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 widespread 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.<sup>6</sup> 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 encoded by a single gene.<sup>61,62</sup> This strategy appears to be employed by desert soil methanogens.<sup>22</sup>

Alternatively, microbes may use pathways not affected by oxygen (Figure 2). Karl et al.<sup>23</sup> suggest that microbes in the



**Figure 2.** Known and hypothetical methanogenesis pathways. EC numbers for catalyzing enzymes marked in green are found in genome annotation of nonmethanogenic 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’ unpublished data marked with an asterisk). Pathways known to require anoxic environments are grouped in black frames. Known and hypothesized pathways that occur under oxic conditions are grouped within solid and dashed red frames, respectively. See the text for details.

equatorial Pacific break down methylphosphonate (MPn) and release methane as a byproduct. The process requires enzymatic cleavage of the C–P bond and is not sensitive to oxygen. The operon for the C–P lyase enzyme PhnJ responsible for breaking the C–P bond<sup>63</sup> is widespread across the bacterial domain.<sup>64</sup> Accordingly, this reaction can be catalyzed by numerous phosphorus-scavenging microorganisms and supports the notion that MPn is a main phosphorus source for microbes in oligotrophic waters.<sup>65,66</sup> While biological phosphonate production is common within the bacterial domain,<sup>67</sup> currently only one methylphosphonate synthase (mpnS) has been identified originating from the marine Thaumarchaeota.<sup>68</sup> These organisms are abundant in the ocean, although they have been reported in some freshwater lakes, as well.<sup>69</sup> Another probable source in freshwater are the Actinobacteria, which produce a large diversity of phosphonate compounds.<sup>70,71</sup> The large abundance of freshwater Actinobacteria coupled with known C–P lyase activity of cyanobacteria<sup>72</sup> may explain the correlation between oxic methane formation and cyanobacteria bloom in Lake Stechlin.<sup>12</sup> Damm et al.<sup>25</sup> suggest that Arctic microbes metabolize dimethylsulfoniopropionate (DMSP) (requiring enzymatic cleavage of the C–S bond) for energy production and release methane as a byproduct, which would require a final step of methyl reduction. However, the methyl reductase Mcr gene complex has not been found in any nonmethanogenic genome and has no known structural homologue in Bacteria. To allow the process to occur in oxic water, Damm et al.<sup>73</sup> theorize that DMSP-utilizing bacteria maintain an anoxic cytoplasm through respiration, although empirical evidence is still missing.

From an energetic standpoint, discarding methane as a byproduct is hardly favorable; 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,<sup>23,25</sup> which are common among heterotrophic microbes capable of metabolizing C-1 compounds.<sup>74</sup> A comparative genomics analysis shows that the majority of enzymes in the various methanogenic pathways are present in nonmethanogenic organisms, including Bacteria (Figure 2). This along with the presence of several C1 carriers (tetrahydrofolate and tetrahydromethanopterin)<sup>75</sup> among Bacteria allows us to speculate that upon demethylation of C-1 compounds, the methyl group bound to a C-1 carrier or an unknown Coenzyme-M homologue is reduced to methane by cellular reductases, for which the methyl reductase function has not been identified (Figure 2). Alternative sources of reducing power potentially include (1) electron bifurcation that has been described for anaerobic methanogenesis<sup>76</sup> but not yet for oxic methane production and (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 bypassing the involvement of heterotrophic microbes.<sup>77</sup> Organosulfur compounds such as methionine, dimethyl sulfoxide, and DMSP are commonly produced by algae. It has been reported that, under ambient atmospheric conditions, several organosulfur compounds can be chemically converted to methane.<sup>9</sup> 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

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

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.<sup>3,4</sup> 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 on the different precursor compounds and pathways. (2) Further investigation of the fate of this novel methane source, including water-to-air exchange and internal consumption via methanotrophy, is warranted. (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 the future climate.

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

We thank Mina Bizic-Ionescu for providing methane data from the LakeLab mesocosms in Lake Stechlin and Dominic Vachon for helping with graphic for the table of contents. K.W.T. was supported by a Humboldt Fellowship for Experienced Researchers (Germany). D.I. and H.-P.G. were supported by a grant from the German Science Foundation (AquaMeth Project GR1540/21-1). Prof. F. Keppler provided valuable comments on an earlier draft.

## ■ REFERENCES

- (1) IPCC. Climate change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. United Nations: Geneva, 2013.
- (2) Reeburgh, W. S. Oceanic Methane Biogeochemistry. *Chem. Rev.* **2007**, *107*, 486–513.
- (3) Conrad, R. The Global Methane Cycle: Recent Advances in Understanding The Microbial Processes Involved. *Environ. Microbiol. Rep.* **2009**, *1*, 285–292.
- (4) Kirschke, S.; Bousquet, P.; Ciais, P.; Saunoy, M.; Canadell, J. G.; Dlugokencky, E. J.; Bergamaschi, P.; Bergmann, D.; Blake, D. R.; Bruhwiler, L.; et al. Three Decades of Global Methane Sources and Sinks. *Nat. Geosci.* **2013**, *6*, 813–823.
- (5) Jarrell, K. F. Extreme Oxygen Sensitivity in Methanogenic Archaeobacteria. *BioScience* **1985**, *35*, 298–302.
- (6) Madigan, M. T.; Martinko, J. M.; Bender, K. S.; Buckley, D. H.; Stahl, D. A.; Brock, T. *Brock Biology of Microorganisms*; Benjamin Cummings: San Francisco, 2014.

- (7) Lenhart, K.; Bunge, M.; Ratering, S.; Neu, T. R.; Schüttmann, I.; Greule, M.; Kammann, C.; Schnell, S.; Müller, C.; Zorn, H.; Keppler, F. Evidence of Methane Production by Saprotrophic Fungi. *Nat. Commun.* **2012**, *3*, 1046.
- (8) Keppler, F.; Hamilton, J. T.; Braß, M.; Röckmann, T. Methane Emissions from Terrestrial Plants under Aerobic Conditions. *Nature* **2006**, *439*, 187–191.
- (9) Althoff, F.; Benzing, K.; Comba, P.; McRoberts, C.; Boyd, D. R.; Greiner, S.; Keppler, F. Abiotic Methanogenesis from Organosulphur Compounds under Ambient Conditions. *Nat. Commun.* **2014**, *5*, 4205.
- (10) Ghyczy, M.; Torday, C.; Kaszaki, J.; Szabó, A.; Czöbel, M.; Boros, M. Hypoxia-Induced Generation of Methane in Mitochondria and Eukaryotic Cells- An Alternative Approach to Methanogenesis. *Cell. Physiol. Biochem.* **2008**, *21*, 251–258.
- (11) Jugold, A.; Althoff, F.; Hurkuck, M.; Greule, M.; Lenhart, K.; Lelieveld, J.; Keppler, F. Non-microbial Methane Formation in Oxidic Soils. *Biogeosciences* **2012**, *9*, 5291–5301.
- (12) Grossart, H. P.; Frindt, K.; Dziallas, C.; Eckert, W.; Tang, K. W. Microbial Methane Production in Oxygenated Water Column of An Oligotrophic Lake. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 19657–19661.
- (13) Murase, J.; Sakai, Y.; Kametani, A.; Sugimoto, A. Dynamics of Methane in Mesotrophic Lake Biwa, Japan. *Ecol. Res.* **2005**, *20*, 377–385.
- (14) Hofmann, H.; Federwisch, L.; Peeters, F. Wave-induced Release of Methane: Littoral Zones as a Source of Methane in Lakes. *Limnol. Oceanogr.* **2010**, *55*, 1990–2000.
- (15) Oremland, R. S. Methanogenic Activity in Plankton Samples and Fish Intestines: A Mechanism for In Situ Methanogenesis in Oceanic Surface Waters. *Limnol. Oceanogr.* **1979**, *24*, 1136–1141.
- (16) Sieburth, J. M. Contrary Habitats for Redox-Specific Processes: Methanogenesis in Oxidic Waters and Oxidation in Anoxic Waters. In *Microbes in the Sea*; Sleigh, M. A., Ed.; Ellis Horwood: Chichester, U.K., 1987.
- (17) DeAngelis, M. A.; Lee, C. Methane Production during Zooplankton Grazing on Marine Phytoplankton. *Limnol. Oceanogr.* **1994**, *39*, 1298–1308.
- (18) Kirschbaum, M. U.; Bruhn, D.; Etheridge, D. M.; Evans, J. R.; Farquhar, G. D.; Gifford, R. M.; Paul, K. I.; Winters, A. J. A Comment on the Quantitative Significance of Aerobic Methane Release by Plants. *Funct. Plant Biol.* **2006**, *33*, 521–530.
- (19) Dueck, T. A.; De Visser, R.; Poorter, H.; Persijn, S.; Gorissen, A.; De Visser, W.; Schapendonk, A.; Verhagen, J.; Snel, J.; Harren, F. J. M.; et al. No Evidence for Substantial Aerobic Methane Emission by Terrestrial Plants: a <sup>13</sup>C-labelling Approach. *New Phytol.* **2007**, *175*, 29–35.
- (20) Wang, Z. P.; Chang, S. X.; Chen, H.; Han, X. G. Widespread Non-Microbial Methane Production by Organic Compounds and the Impact of Environmental Stresses. *Earth-Sci. Rev.* **2013**, *127*, 193–202.
- (21) Liu, J.; Chen, H.; Zhu, Q.; Shen, Y.; Wang, X.; Wang, M.; Peng, C. A Novel Pathway of Direct Methane Production and Emission by Eukaryotes Including Plants, Animals and Fungi: An Overview. *Atmos. Environ.* **2015**, *115*, 26–35.
- (22) Angel, R.; Matthies, D.; Conrad, R. Activation of Methanogenesis in Arid Biological Soil Crusts Despite the Presence of Oxygen. *PLoS One* **2011**, *6*, e20453.
- (23) Karl, D. M.; Beversdorf, L.; Björkman, K. M.; Church, M. J.; Martinez, A.; Delong, E. F. Aerobic Production of Methane in the Sea. *Nat. Geosci.* **2008**, *1*, 473–478.
- (24) Del Valle, D. A.; Karl, D. M. Aerobic Production of Methane from Dissolved Water-Column Methylphosphonate and Sinking Particles in the North Pacific Subtropical Gyre. *Aquat. Microb. Ecol.* **2014**, *73*, 93–105.
- (25) Damm, E.; Helmke, E.; Thoms, S.; Schauer, U.; Nöthig, E.; Bakker, K.; Kiene, R. P. Methane Production in Aerobic Oligotrophic Surface Water in the Central Arctic Ocean. *Biogeosciences* **2010**, *7*, 1099–1108.
- (26) Damm, E.; Rudels, B.; Schauer, U.; Mau, S.; Dieckmann, G. Methane Excess in Arctic Surface Water- Triggered by Sea Ice Formation and Melting. *Sci. Rep.* **2015**, *5*, 16179.
- (27) Damm, E.; Thoms, S.; Beszczynska-Möller, A.; Nöthig, E. M.; Kattner, G. Methane Excess Production in Oxygen-rich Polar Water and a Model of Cellular Conditions for this Paradox. *Polar Sci.* **2015**, *9*, 327–334.
- (28) Scranton, M. I.; Brewer, P. G. Occurrence of Methane in the Near-Surface Waters of the Western Subtropical North-Atlantic. *Deep-Sea Res.* **1977**, *24*, 127–138.
- (29) Conrad, R.; Seiler, W. Methane and Hydrogen in Seawater (Atlantic Ocean). *Deep-Sea Res., Part A* **1988**, *35*, 1903–1917.
- (30) Ward, B. B.; Kilpatrick, K. A. Methane Oxidation Associated with Mid-depth Methane Maxima in the Southern California Bight. *Cont. Shelf Res.* **1993**, *13*, 1111–1122.
- (31) Watanabe, S.; Higashitani, N.; Tsurushima, N.; Tsunogai, S. Methane in the Western North Pacific. *J. Oceanogr.* **1995**, *51*, 39–60.
- (32) Tilbrook, B. D.; Karl, D. M. Methane Sources, Distributions and Sinks from California Coastal Waters to the Oligotrophic North Pacific Gyre. *Mar. Chem.* **1995**, *49*, 51–64.
- (33) Jayakumar, D. A.; Naqvi, S. W. A.; Narvekar, P. V.; George, M. D. Methane in Coastal and Offshore Waters of the Arabian Sea. *Mar. Chem.* **2001**, *74*, 1–13.
- (34) Rehder, G.; Brewer, P. W.; Peltzer, E. T.; Friederich, G. Enhanced Lifetime of Methane Bubble Streams within the Deep Ocean. *Geophys. Res. Lett.* **2002**, *29*, 21-1–21-4.
- (35) McGinnis, D. F.; Greinert, J.; Artemov, Y.; Beaubien, S. E.; Wüest, A. N. D. A. Fate of Rising Methane Bubbles in Stratified Waters: How Much Methane Reaches the Atmosphere? *J. Geophys. Res.* **2006**, *111*, C09007.
- (36) Sasakawa, M.; Tsunogai, U.; Kameyama, S.; Nakagawa, F.; Nojiri, Y.; Tsuda, A. Carbon Isotopic Characterization for the Origin of Excess Methane in Subsurface Seawater. *J. Geophys. Res.* **2008**, *113*, C030112.
- (37) Damm, E.; Thoms, S.; Kattner, G.; Beszczynska-Möller, A.; Nöthig, E. M.; Stimac, I. Coexisting Methane and Oxygen Excesses in Nitrate-limited Polar Water (Fram Strait) during Ongoing Sea Ice Melting. *Biogeosci. Diss.* **2011**, *8*, 5179–5195.
- (38) Vereshchagina, O. F.; Korovitskaya, E. V.; Mishukova, G. I. Methane in Water Columns and Sediments of the North Western Sea of Japan. *Deep Sea Res., Part II* **2013**, *86–87*, 25–33.
- (39) Florez-Leiva, L.; Damm, E.; Fariás, L. Methane Production Induced by Dimethylsulfide in Surface Water of an Upwelling Ecosystem. *Prog. Oceanogr.* **2013**, *112–113*, 38–48.
- (40) Rudy, J. W. M.; Furutani, A.; Flett, R. J.; Hamilton, R. D. Factors Controlling Methane Oxidation in Shield Lakes: The Role of Nitrogen Fixation and Oxygen Concentration. *Limnol. Oceanogr.* **1976**, *21*, 357–364.
- (41) Bedard, C.; Knowles, R. Some Properties of Methane Oxidation in a Thermally Stratified Lake. *Can. J. Fish. Aquat. Sci.* **1997**, *54*, 1639–1645.
- (42) Sundh, I.; Bastviken, D.; Tranvik, L. J. Abundance, Activity, and Community Structure of Pelagic Methane-oxidizing Bacteria in Temperate Lakes. *Appl. Environ. Microbiol.* **2005**, *71*, 6746–6752.
- (43) Bastviken, D.; Cole, J. J.; Pace, M. L.; Van de Bogert, M. C. Fates of Methane from Different Lake Habitats: Connecting Whole-Lake Budgets and CH<sub>4</sub> Emissions. *J. Geophys. Res.* **2008**, *113*, G02024.
- (44) Juutinen, S.; Rantakari, M.; Kortelainen, P.; Huttunen, J. T.; Larmola, T.; Alm, J.; Silvola, J.; Martikainen, P. J. Methane Dynamics in Different Boreal Lake Types. *Biogeosciences* **2009**, *6*, 209–223.
- (45) Vagle, S.; Hume, J.; McLaughlin, F.; MacIsaac, E.; Shortreed, K. A Methane Bubble Curtain in Meromictic Sakinaw Lake, British Columbia. *Limnol. Oceanogr.* **2010**, *55*, 1313–1326.
- (46) Bogard, M. J.; del Giorgio, P. A.; Boutet, L.; Chaves, M. C. G.; Prairie, Y. T.; Merante, A.; Derry, A. M. Oxidic Water Column Methanogenesis as a Major Component of Aquatic CH<sub>4</sub> Fluxes. *Nat. Commun.* **2014**, *5*, 5350.

- (47) Tang, K. W.; McGinnis, D. F.; Frindte, K.; Brüchert, V.; Grossart, H. P. Paradox Reconsidered: Methane Oversaturation in Well-Oxygenated Lake Waters. *Limnol. Oceanogr.* **2014**, *59*, 275–284.
- (48) Bleses, J.; Niemann, H.; Erne, M.; Zopfi, J.; Schubert, C. J.; Lehmann, M. F. Spatial Variations in Surface Water Methane Saturation and Emission in Lake Lugano, Southern Switzerland. *Aquat. Sci.* **2015**, *77*, 535–545.
- (49) DeAngelis, M. A.; Lilley, M. D. Methane in Surface Waters of Oregon Estuaries and Rivers. *Limnol. Oceanogr.* **1987**, *32*, 716–722.
- (50) Verpoorter, C.; Kutser, T.; Seekell, D. A.; Tranvik, L. J. A Global Inventory of Lakes Based on High-resolution Satellite Imagery. *Geophys. Res. Lett.* **2014**, *41*, 6396–6402.
- (51) Yoshikawa, C.; Hayashi, E.; Yamada, K.; Yoshida, O.; Toyoda, S.; Yoshida, N. Methane Sources and Sinks in the Subtropical South Pacific Along 17°S as Traced by Stable Isotope Ratios. *Chem. Geol.* **2014**, *382*, 24–31.
- (52) Murase, J.; Sugimoto, A. Inhibitory Effect of Light on Methane Oxidation in the Pelagic Water Column of a Mesotrophic Lake (Lake Biwa, Japan). *Limnol. Oceanogr.* **2005**, *50*, 1339–1343.
- (53) MacIntyre, S.; Jonsson, A.; Jansson, M.; Aberg, J.; Turney, D. E.; Miller, S. D. Buoyancy Flux, Turbulence, and the Gas Transfer Coefficient in a Stratified Lake. *Geophys. Res. Lett.* **2010**, *37*, L24604.
- (54) McGinnis, D. F.; Kirillin, G.; Tang, K. W.; Flury, S.; Bodmer, P.; Engelhardt, C.; Casper, C.; Grossart, H. P. Enhancing Surface Methane Fluxes from an Oligotrophic Lake: Exploring the Microbubble Hypothesis. *Environ. Sci. Technol.* **2015**, *49*, 873–880.
- (55) Encinas Fernandez, J.; Peeters, F.; Hofmann, H. Importance of the Autumn Overturn and Anoxic Conditions in the Hypolimnion for the Annual Methane Emissions from a Temperate Lake. *Environ. Sci. Technol.* **2014**, *48*, 7297–7304.
- (56) Prairie, Y.; del Giorgio, P. A. New Pathway of Freshwater Methane Emissions and the Putative Importance of Microbubbles. *Inland Waters* **2013**, *3*, 311–320.
- (57) Bastviken, D.; Tranvik, L. J.; Downing, J. A.; Crill, P. M.; Enrich-Prast, A. Freshwater Methane Emissions Offset the Continental Carbon Sink. *Science* **2011**, *331*, 50.
- (58) Cole, J. J.; Caraco, N. F. Atmospheric Exchange of Carbon Dioxide in a Low-wind Oligotrophic Lake Measured by the Addition of SF<sub>6</sub>. *Limnol. Oceanogr.* **1998**, *43*, 647–656.
- (59) Crusius, J.; Wanninkhof, R. Gas Transfer Velocities Measured at Low Wind Speed over a Lake. *Limnol. Oceanogr.* **2003**, *48*, 1010–1017.
- (60) Eugster, W.; Kling, G.; Jonas, T.; McFadden, J. P.; Wüest, A.; MacIntyre, S.; Chapin, F. S. CO<sub>2</sub> Exchange between Air and Water in an Arctic Alaskan and Midlatitude Swiss Lake: Importance of Convective Mixing. *J. Geophys. Res.* **2003**, *108*, D12.
- (61) Haas, A.; Brehm, K.; Kreft, J.; Goebel, W. Cloning, Characterization, and Expression in *Escherichia coli* of a Gene Encoding *Listeria seeligeri* Catalase, a Bacterial Enzyme Highly Homologous to Mammalian Catalases. *J. Bacteriol.* **1991**, *173*, 5159–5167.
- (62) Brunder, W.; Schmidt, H.; Karch, H. KatP, a Novel Catalase-peroxidase Encoded by the Large plasmid of Enterohaemorrhagic *Escherichia coli* O157:H7. *Microbiology* **1996**, *142*, 3305–3315.
- (63) Kamat, S. S.; Williams, H. J.; Dangott, L. J.; Chakrabarti, M.; Rauschel, F. M. The Catalytic Mechanism For Aerobic Formation Of Methane By Bacteria. *Nature* **2013**, *497*, 132–136.
- (64) Villarreal-Chiu, J. F.; Quinn, J. P.; McGrath, J. W. The Genes and Enzymes of Phosphonate Metabolism by Bacteria, and Their Distribution in the Marine Environment. *Front. Microbiol.* **2012**, *3*, 19.
- (65) Dyhrman, S. T.; Benitez-Nelson, C. R.; Orchard, E. D.; Haley, S. T.; Pellechia, P. J. A. A Microbial Source of Phosphonates in Oligotrophic Marine Systems. *Nat. Geosci.* **2009**, *2*, 696–699.
- (66) Carini, P.; White, A. E.; Campbell, E. O.; Giovannoni, S. J. Methane Production by Phosphate-starved SAR11 Chemoheterotrophic Marine Bacteria. *Nat. Commun.* **2014**, *5*, 4346.
- (67) Yu, X.; Doroghazi, J. R.; Janga, S. C.; Zhang, J. K.; Circello, B.; Griffin, B. M.; Labeda, D. P.; Metcalf, W. W. Diversity and Abundance of Phosphonate Biosynthetic Genes in Nature. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 20759–20764.
- (68) Metcalf, W. W.; et al. Synthesis of Methylphosphonic Acid by Marine Microbes: A Source for Methane in the Aerobic Ocean. *Science* **2012**, *337*, 1104–1107.
- (69) Callieri, C.; Hernández-Avilés, S.; Salcher, M. M.; Fontaneto, D.; Bertoni, R. Distribution Patterns and Environmental Correlates of Thaumarchaeota Abundance in Six Deep Subalpine Lakes. *Aquat. Sci.* **2016**, *78*, 215–225.
- (70) Ju, K.-S.; Gao, J.; Doroghazi, J. R.; Wang, K.-K. A.; Thibodeaux, C. J.; Li, S.; Metzger, E.; Fudala, J.; Su, J.; Zhang, J. K.; et al. Discovery of Phosphonic Acid Natural Products by Mining the Genomes of 10,000 Actinomycetes. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 12175–12180.
- (71) White, A. K.; Metcalf, W. W. Microbial Metabolism of Reduced Phosphorus Compounds. *Annu. Rev. Microbiol.* **2007**, *61*, 379–400.
- (72) Gomez-Garcia, M.; Davison, M.; Blain-Hartnung, M.; Grossman, A. R.; Bhaya, D. Alternative Pathways for Phosphonate Metabolism in Thermophilic Cyanobacteria from Microbial Mats. *ISME J.* **2011**, *5*, 141–149.
- (73) Damm, E.; Thoms, S.; Beszczynska-Möller, A.; Nöthig, E. M.; Kattner, G. Methane Excess Production in Oxygen-rich Polar Water and a Model of Cellular Conditions for this Paradox. *Polar Sci.* **2015**, *9*, 327–334.
- (74) Colby, J.; Dalton, H.; Whittenbury, R. Biological and Biochemical Aspects of Microbial Growth on C1 Compounds. *Annu. Rev. Microbiol.* **1979**, *33*, 481–517.
- (75) Chistoserdova, L.; Vorholt, J. A.; Thauer, R. K.; Lidstrom, M. E. C-1 Transfer Enzymes and Coenzymes Linking Methylophilic Bacteria and Methanogenic Archaea. *Science* **1998**, *281*, 99–102.
- (76) Buckel, W.; Thauer, R. K. Energy Conservation via Electron Bifurcating Ferredoxin Reduction and Proton/Na<sup>+</sup> Translocating Ferredoxin Oxidation. *Biochim. Biophys. Acta, Bioenerg.* **2013**, *1827*, 94–113.
- (77) Lenhart, K.; Klintzsch, T.; Langer, G.; Nehrke, G.; Bunge, M.; Schnell, S.; Keppler, F. Evidence of Methane Production by Marine Algae (*Emiliania huxleyi*) and its Implication for the Methane Paradox in Oxidic Waters. *Biogeosci. Diss.* **2015**, *12*, 20323–20360.
- (78) Paerl, H. W.; Huisman, J. Climate Change: A Catalyst for Global Expansion of Harmful Cyanobacterial Blooms. *Environ. Microbiol. Rep.* **2009**, *1*, 27–37.
- (79) MacKay, M. D.; Neale, P. J.; Arp, C. D.; De Senerpont Domis, L. N.; Fang, X.; Gal, G.; Jöhnk, K. D.; Kirillin, G.; Lenters, J. D.; Litchman, E.; et al. Modeling Lakes and Reservoirs in the Climate System. *Limnol. Oceanogr.* **2009**, *54*, 2315–2329.