Aerobic production of methane in the sea

DAVID M. KARL[1]*, LUCAS BEVERSDORF[1], KARIN M. BJÖRKMAN[1], MATTHEW J. CHURCH[1], ASUNCION MARTINEZ[2] AND EDWARD F. DELONG[2]

[1]University of Hawaii, Honolulu, Hawaii 96822, USA
[2]Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
*e-mail: dkarl@hawaii.edu

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Methane is a potent greenhouse gas that has contributed approximately 20% to the Earth's warming since pre-industrial times. The world's oceans are an important source of methane, comprising 1–4% of annual global emissions. But despite its global significance, oceanic methane production is poorly understood. In particular, methane concentrations in the surface waters of most of the world's oceans are supersaturated with respect to atmospheric concentrations, but the origin of this methane, which has been thought to be produced exclusively in anaerobic environments, is not known. Here, we measure methane production in seawater samples amended with methylphosphonate, an organic, phosphorus-containing compound. We show that methane is produced aerobically as a by-product of methylphosphonate decomposition in phosphate-stressed waters. Methylphosphonate decomposition, and thus methane production, may be enhanced by the activity of nitrogen-fixing microorganisms. We suggest that aerobic marine methane production will be sensitive to the changes in water-column stratification and nutrient limitation that are likely to result from greenhouse-gas-induced ocean warming.

The oceanic methane cycle consists of a complex set of contemporary, mostly microbiological, reactions involving methane production (methanogenesis) and methane consumption (methanotrophy) superimposed on an equally complex set of geological processes including hydrothermal vents, cold seeps, mud volcanoes and methane clathrate systematics, to name a few examples[1,2]. Throughout most of the oceanic water column, methane is undersaturated with respect to atmospheric concentrations as a consequence of net microbial oxidation. However, methane concentrations in near-surface waters throughout much of the global ocean are 5–75% supersaturated with respect to the atmosphere, implying local methanogenesis and a net flux from the ocean to the atmosphere[3–6]. Because the surface ocean is also saturated or slightly supersaturated with respect to atmospheric oxygen, which should not favour methanogenesis, the as-yet-unexplained methane supersaturation has been termed the 'oceanic methane paradox'[7].

OCEANIC PHOSPHORUS CYCLE

Phosphorus (P) is an essential macronutrient for all living organisms, and during growth P is incorporated into a broad spectrum of organic compounds[8]. On cell death and autolysis, these P-containing cellular materials are differentially remineralized and a portion is used to fuel another round of cell growth. There are two major forms of organic-P compounds in the ocean: phosphate esters and phosphonates. Phosphonates are characterized by a carbon–phosphorus (C–P) bond rather than the more common carbon–oxygen–phosphorus (C–O–P) ester link that is found in biomolecules such as AMP and DNA (ref. 9). Phosphonates have been identified as components of marine phytoplankton, sinking particulate organic matter and high-molecular-weight (>1,000 daltons) dissolved-organic-matter pools in the sea[9–12], implying a marine source. However, the molecular composition, concentrations and turnover rates of phosphonates in sea water, especially the low-molecular-weight pools, are presently unknown. Methylphosphonate (MPn), the simplest of the C–P bonded compounds, may be both a precursor for the biosynthesis of more complex phosphonate molecules and a partial degradation product thereof. In *Escherichia coli*, *Pseudomonas* and many other bacteria, MPn can be used as a sole source of P during aerobic growth[13–15]. During MPn utilization, methane is quantitatively released, whereas P is incorporated into new cell mass[16].

THE PHOSPHONATE–METHANE HYPOTHESIS

We hypothesize that MPn cycling (its coupled production and decomposition) is a pathway for the aerobic formation of methane in marine ecosystems. The aerobic formation of methane in the sea may represent the 'missing' source term needed to reconcile the observed methane supersaturations that sustain a net flux of methane from the ocean to the atmosphere.

We initially tested this hypothesis by conducting a series of experiments to determine the presence and activity of the putative aerobic methanogens and to characterize the physiological controls on methane production. Seawater samples for these experiments were collected at the Hawaii Ocean Time-series (HOT) Station ALOHA (22°45′ N, 158° W). Previous investigations in this region reported high concentrations of methane and oxygen as well as low concentrations of inorganic nutrients, including both nitrate and phosphate (Table 1). These conditions characterize subtropical ocean gyres worldwide.

The first experiment was designed to detect the presence of heterotrophic marine bacteria that grew using MPn as the sole source of P, and to establish whether methane was produced as a by-product of this metabolic pathway. The results documented the following (Fig. 1a,b; Table 2): (1) unfiltered surface seawater samples amended with glucose plus nitrate

Table 1 Typical environmental conditions in the upper water column of the North Pacific subtropical gyre.

Parameters	Values*
Methane	
Concentration	2–4 nmol l^{-1}
Relative air saturation	105–175%
Sea-to-air flux	0.9–3.5 μmol m^{-2} d^{-1}
Oxygen	
Concentration	205–215 μmol l^{-1}
Relative air saturation	100–104%
Gross primary production	0.6–1.2 μmol O_2 l^{-1} d^{-1}
Respiration	0.5–1.0 μmol O_2 l^{-1} d^{-1}
Nutrients	
Nitrate	1–5 nmol l^{-1}
Phosphate	20–100 nmol l^{-1}
Dissolved organic carbon	80–100 μmol l^{-1}
Dissolved organic nitrogen	5–6 μmol l^{-1}
Dissolved organic phosphorus	0.2–0.3 μmol l^{-1}
Microorganisms	
Chlorophyll *a*	0.05–0.15 μg l^{-1}
Prochlorococcus	2–3 × 10^8 cells l^{-1}
Synechococcus	1–4 × 10^6 cells l^{-1}
Heterotrophic bacteria†	6–7 × 10^8 cells l^{-1}
Biological Production	
Photosynthesis (light ^{14}C-bicarbonate incorporation)	0.5–2 μmol C l^{-1} d^{-1}
Heterotrophic bacterial production (dark ^{3}H-leucine incorporation)	0.07–0.28 μmol C l^{-1} d^{-1}
Phosphate uptake	3–4 nmol P l^{-1} d^{-1}
Dissolved organic phosphorus uptake	1–5 nmol P l^{-1} d^{-1}

*Obtained from a variety of published sources and from the HOT program database (http://hahana.soest.hawaii.edu).

†Heterotrophic bacteria include all non-pigmented picoplankton.

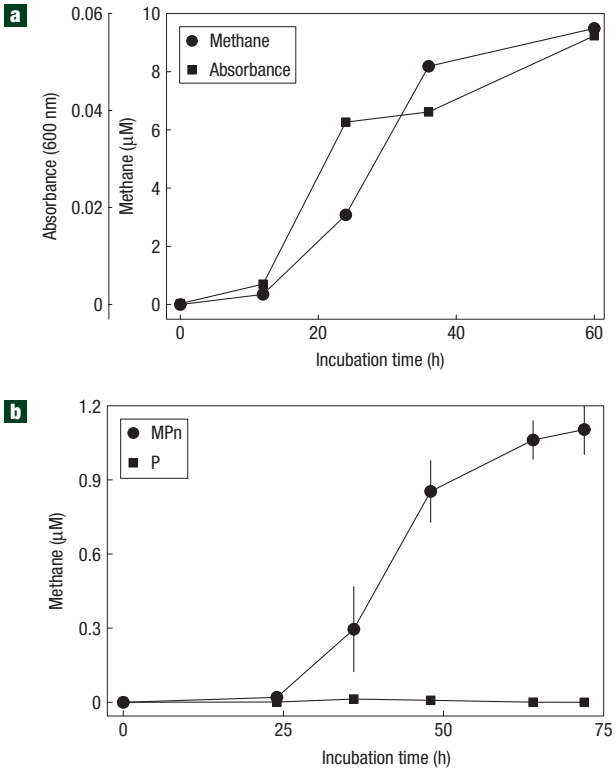

Figure 1 Aerobic production of methane from MPn in glucose-plus-nitrate-amended surface seawater samples collected from Station ALOHA. **a**, Methane and biomass accumulation (absorbance at 660 nm) during aerobic growth following enrichment with 1 mM glucose / 160 μM nitrate / 10 μM MPn. **b**, Methane accumulation during aerobic growth in a more dilute enrichment medium containing 100 μM glucose /16 μM nitrate and 1 μM of either MPn or phosphate (P) added as the sole P source, as indicated. Error bars are 1 standard deviation of the mean; $n = 3$.

rapidly consumed exogeneous MPn and produced methane under aerobic incubation conditions, indicating the presence of actively growing MPn-degrading microorganisms; (2) the rate of methane accumulation in the cultures tracked particulate matter accumulation and documented a stoichiometric conversion of MPn to methane during growth; and (3) methane was not produced in replicate glucose-plus-nitrate-amended control samples that received an equivalent spike of phosphate in place of MPn (Fig. 1b), or in filtered (0.2 μm) treatments (data not shown).

In the second set of experiments, we evaluated nutrient controls on methane production by varying either the glucose/nitrate/MPn or the phosphate/MPn ratios. As before, nutrient-amended surface sea water from Station ALOHA rapidly produced methane following MPn addition. The production of methane from MPn was regulated by nitrate concentration in glucose-sufficient treatments (Fig. 2a); when growth ceased, methane production also ceased. The utilization of MPn (methane production) was downregulated, but not totally eliminated, by the simultaneous addition of phosphate (Fig. 2b). Phosphate is often considered to be the preferred P substrate for microbial growth in the sea. The yields of methane were greatest for the 1 μM MPn-only treatment and decreased with increasingly greater proportions of phosphate (Fig. 2b). For example, samples initially receiving equimolar MPn and phosphate (1 μM each, total $P = 2$ μM) produced methane at approximately 10% of the rate of the MPn-only (1 μM) treatment. These results suggest that whereas MPn and phosphate are metabolized concurrently, phosphate uptake is favoured and that dilution of the MPn pool with phosphate results in a systematic reduction in the yield of methane (a relative decrease in MPn utilization). This result would be predicted by mass action if both substrates were readily available for assimilation at these concentrations. When a second amendment of glucose plus nitrate was added to the phosphate-plus-MPn-enriched, methane-underproducing treatments, there was an immediate and predictable phosphate-MPn-scaled enhancement in methane production rate (Fig. 2b). These results confirm that marine microbes, at least at the community level, are able to switch rapidly from one P substrate to the other, and that initial growth on phosphate does not alter the potential for subsequent MPn-supported methane production when phosphate becomes limiting. From the design of these experiments, we cannot distinguish between simultaneous utilization of phosphate and MPn in one organism versus partitioning of P resources among different members of the microbial assemblage. Nevertheless, the cumulative production of methane seems to be regulated by phosphate when both organic carbon and nitrogen are available in excess.

Several pathways of phosphonate utilization have been characterized in bacteria. Of these, only the C–P lyase pathway results in utilization of MPn as a substrate with concomitant release of methane[14,15,17]. In *E. coli*, the C–P lyase pathway is encoded by a cluster of 14 genes (*phnC–phnP*), the expression of which is an integral component of the *Pho*-regulon, ultimately controlled by phosphate availability[14,15,17]. Analyses of sequenced bacterial genomes have shown that the C–P lyase pathway is found in

Table 2 Carbon, nitrogen, phosphorus and methane budgets of surface-seawater-amendment incubation experiment. (Surface sea water amended with 100 μM glucose, 16 μM nitrate and approximately 1 μM MPn.)

Incubation time	Methane	Dissolved organic carbon	Nitrate	Particulate matter (μM)*			C/N/P* ratio
(h)	(μM)	(μM)	(μM)	C	N	P	
0	0	578	16.2	4.6	0.74	0.03	153:25:1
36	0.20	533	12.5	25.4	3.74	0.16	159:23:1
48	0.85	361	0.6	108.2	12.39	0.53	204:23:1
64	1.06	244	<0.01	129.9	14.24	0.54	241:26:1
72	1.10	228	<0.01	125.7	12.58	0.50	251:25:1

*The particulate-P determinations do not include polyphosphates and should be taken as a lower bound on total cell-P.

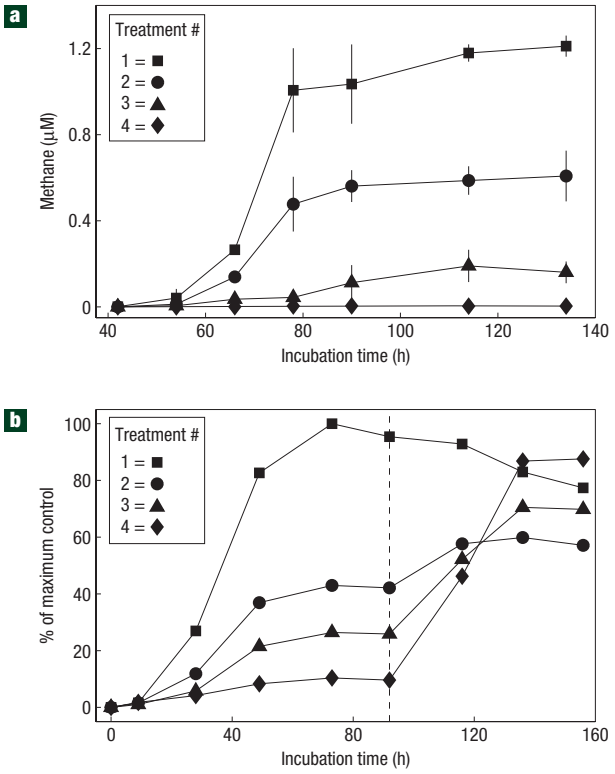

Figure 2 Methane accumulation in surface seawater samples collected from Station ALOHA. a, #1–100 μM glucose/16 μM nitrate/1.2 μM MPn, #2–50 μM glucose/8 μM nitrate/1.2 μM MPn, #3–25 μM glucose/4 μM nitrate/1 μM MPn, #4–100 μM glucose/16 μM nitrate/1.2 μM phosphate (negative control). **b**, Methane accumulation, expressed as per cent of maximum in the MPn-only positive control, amended with 100 μM glucose, 16 μM nitrate and varying proportions of P. #1–1 μM MPn only (total P = 1.0 μM), #2–1 μM MPn plus 0.5 μM phosphate (total P = 1.5 μM), #3–1 μM MPn plus 0.75 μM phosphate (total P = 1.75 μM), #4–1 μM MPn plus 1 μM phosphate (total P = 2.0 μM). At 96 h in the incubation period (dashed vertical line), a second aliquot of 100 μM glucose plus 16 μM nitrate was added to each treatment.

diverse microorganisms including members of the Proteobacteria, Firmicutes, Bacteroidetes, Chloroflexi and Cyanobacteria[15,17]. We used the IMG engine (http://img.jgi.doe.gov) to query the numerous marine microbial genomes that have recently become available and found complete orthologue C–P lyase pathways in 35 marine isolates. We also searched a microbial community genome database (~65 Mbp total) prepared from Station ALOHA (ref. 18) and found numerous examples of known MPn-utilization pathway genes. The most abundant were putative phosphonate ABC transporter genes (*phnC*, *phnD* and *phnE*), encoding proteins with high amino acid similarity (37–70%) to homologues from Alphaproteobacteria, Gammaproteobacteria, Cyanobacteria and Firmicutes. C–P lyase components (*phnG*, *phnH*, *phnI*, *phnK* and *phnL*) were also found in the Station ALOHA metagenomic database. A larger data set from a study conducted in the Sargasso Sea[19] (their samples #2–7, 1.1 Gbp total) revealed significant representation of genes encoding C–P lyase components (Table 3). When these gene occurrences were normalized to the abundance of a single-copy gene[20] (*recA*) recovered in the same data set, the results indicated that a significant percentage of microorganisms in the Sargasso Sea sample contain C–P lyase pathway genes (Table 3). The majority of the C–P lyase genes (89%) in the Sargasso Sea habitat bore highest similarity to homologues from *Roseobacter*-related members of the Alphaproteobacteria. We conclude that there exists significant representation of known MPn-utilization pathways in marine picoplankton, and that its expression may account for the aerobic methane production that we observed.

Dyhrman *et al.*[21] recently reported the expression of phosphonate metabolism genes (*phnD* and *phnJ*) in *Trichodesmium erythraeum* (IMS 101), a cosmopolitan nitrogen-fixing marine cyanobacterium. The expression of phosphonate-utilization genes was detected in phosphate-deficient, but not in phosphate-replete, laboratory cultures, as well as in selected field samples of *Trichodesmium* collected from the phosphate-deficient (<15 nM) western North Atlantic Ocean. We examined the utilization of MPn by natural assemblages of *Trichodesmium* sampled from the North Pacific gyre near Station ALOHA. Two separate experiments yielded significant methane production for both fusiform ('tufts') and spherical ('puffs') colony morphologies following the addition of MPn, compared with replicate treatments that were amended with phosphate only or MPn plus phosphate (Table 4).

Collectively, our results suggest that aerobic decomposition of MPn may be a source of methane in phosphate-stressed marine habitats worldwide. It should be emphasized that the two previously described, and well-studied, methanogenic pathways (fermentation and dissimilatory carbon dioxide reduction) are inhibited by free oxygen[6]; methanogenesis via carbon dioxide reduction is also inhibited by the nearly inexhaustible pool of sulphate (28 mM) in sea water, which serves as a more energetically favourable terminal electron acceptor in bacterial respiration. Previous investigators have hypothesized that methane may be produced in oxygen- and sulphate-free microenvironments within otherwise well-ventilated surface ocean habitats[22–24]. These studies, and others like them, were based either on laboratory experiments or on incubations of field-collected samples with exogeneous methanogenic substrates under strict anaerobic conditions to document the potential for 'traditional' methanogenesis. One

Table 3 Frequency of C–P lyase genes in the Sargasso Sea shotgun libraries. (Sample collections and analyses described in ref. 19.)

	Number of gene homologues (% of bacterial genomes)*					
Gene	Sargasso 2	Sargasso 3	Sargasso 4	Sargasso 5	Sargasso 6	Sargasso 7
phnG	17 (8)	12 (5)	21 (9)	5 (18)	5 (31)	20 (24)
phnH	21 (10)	12 (5)	18 (8)	4 (14)	7 (44)	27 (32)
phnI	31 (14)	10 (4)	19 (8)	4 (14)	18 (113)	27 (32)
phnJ	13 (6)	6 (3)	9 (4)	1 (4)	12 (75)	20 (24)
phnK	11 (5)	5 (2)	19 (8)	1 (4)	9 (56)	16 (19)
phnL	10 (5)	4 (2)	13 (6)	1 (4)	10 (63)	19 (23)
phnM	25 (5)	9 (4)	23 (10)	2 (7)	21 (131)	31 (37)
recA	217 (100)	228 (100)	231 (100)	28 (100)	16(100)	84 (100)

*Normalized to abundance of a single gene, *recA*, as described in ref. 20.

Table 4 Aerobic production of methane from methylphosphonate-amended seawater samples containing naturally occurring *Trichodesmium* colonies.

Date/Location	Sample*	Treatment†	Methane‡ (nmol l^{-1})
Experiment #1 10 Aug 2007 23°43.9′ N, 159°20.9′ W	Fusiform colonies	+MPn($n=1$)	56.8
	Spherical colonies	+MPn($n=1$)	25.4
	Mixture of fusiform and spherical colonies	−MPn ($n=1$) +MPn ($n=1$) +MPn ($n=1$)	N.D.§ 36.2 28.4
Experiment #2 18 Aug 2007 23°10.2′ N, 159°23.9′ W	Fusiform colonies	+Phosphate ($n=1$) +MPn ($n=1$) +MPn ($n=1$)	N.D. 7.2 14.1
	Spherical colonies	+Phosphate ($n=1$) +MPn ($n=3$)	N.D. 12.2±0.75
	Mixture of colonies	+MPn ($n=3$) +Phosphate/MPn ($n=3$)	70.2±29.3 N.D.
	FSW control	+MPn ($n=1$)	N.D.

*Each sample consisted of pooled (10–20 individual colonies), hand-picked *Trichodesmium* as described in the Methods section. Approximate estimates, based on 100 trichomes per colony, are 0.81–0.92 μmol C $colony^{-1}$ (ref. 38).

†+MPn = 100 nM, +phosphate = 100 nM, +phosphate/MPn = 100 nM of each substrate.

‡Total methane produced in each treatment following a 4 day incubation period (see the Methods section).

§N.D.—not detected; methane production ≤1.5 nmol l^{-1}.

report of methane contained within the interstitial fluids of sinking particles collected from low-nutrient marine habitats provided quantitative data on *in situ* methane production[25]. However, on re-analysis, these results are consistent with MPn decomposition that could occur in particles, in the guts of metazoans or in the surrounding bulk fluid environment irrespective of high oxygen and sulphate concentrations. It has been shown that the relative proportions of phosphate esters and phosphonates in dissolved and particulate organic matter collected at Station ALOHA do not change with water depth, despite significant loss of total organic P (ref. 26). This implies that both compound classes are simultaneously remineralized and that methane may also be produced in subeuphotic zone waters by the metabolism of MPn, thereby providing an extra energy substrate for mesopelagic zone methanotrophs. Failure to detect methane in sinking particles collected in phosphate-sufficient Southern Ocean habitats, and the general absence of near-surface methane supersaturations in these phosphate-replete environments[27] are both predicted from the phosphate control of MPn cycling that we report herein (Figs 1b and 2b).

PHOSPHONATE–METHANE-CLIMATE CONNECTIONS

The global atmospheric inventory of methane has more than doubled since pre-industrial times, but the long-term growth rate slowed significantly and markedly in the late 1990s (refs 28–30). This observed variability is not well understood. Slight temporal imbalances between methane sources and sinks can significantly affect net atmospheric accumulation, even when a given source is a small term in the global budget. On the basis of existing data, annual marine methane emissions ($0.3–1.3 \times 10^{12}$ mol yr^{-1}) comprise approximately 1–4% of the global source[28], and this source term is likely to vary with well-documented decadal and subdecadal climate-based ecosystem variations[31]. Mass balance calculations for the oligotrophic North Pacific gyre based on estimated inputs from *in situ* production and diffusion through the base of the mixed layer and loss due to air–sea gas exchange require a depth-integrated (0–100 m) methane production rate of approximately 2.3 μmol m^{-2} d^{-1} to sustain the observed methane supersaturations at this site[5]. At Station ALOHA, depth-integrated (0–100 m) microbial P assimilation averages 600–650 μmol P m^{-2} d^{-1}; approximately 50% of the required P is derived from sources other than phosphate, including possible contributions from MPn (ref. 32). If only 1–2% of the net organic P flux was cycled through MPn, it would be sufficient to reconcile the oceanic methane paradox.

Our results suggest that the availability of phosphate may regulate methane production in low-nutrient environments. With the possible exception of high-latitude marine environments where phosphate concentrations can exceed 1 μM, surface waters of the global ocean are chronically phosphate-stressed, so the capacity for MPn decomposition is expected under *in situ* conditions.

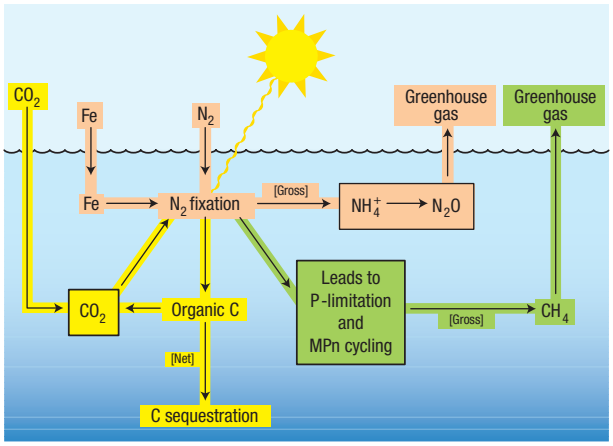

Figure 3 Schematic representation of the hypothetical greenhouse-gas balance for an iron (Fe)-stimulated, nitrogen (N_2)-fixation bloom in the North Pacific gyre. C: carbon; NH_4^+: ammonium; N_2O: nitrous oxide; CO_2: carbon dioxide; CH_4: methane.

Phosphate availability to the well-illuminated upper ocean is controlled largely by the rate of upwelling of nutrient-enriched deep water; open-ocean gyres represent the global minimum in this deep-water resupply.

The cycles of carbon, nitrogen, phosphorus and related bioelements in the sea are inextricably linked through complex networks of microorganisms. Oceanic ecosystems, dominated by microorganisms, have the potential for rapid response to environmental perturbations that could favour opportunistic growth. Nitrogen fixation can relieve an ecosystem of fixed-nitrogen limitation, decouple N and P cycles and promote further phosphate limitation[31,33], and recent global-scale ocean models have shown that the process of nitrogen fixation can amplify biogeochemical responses to dust leading to enhanced carbon dioxide sequestration[34]. Moreover, recent manipulation experiments indicate nitrogen fixation may increase with increasing oceanic carbon dioxide[35], a process that would further exacerbate upper-ocean P limitation. We now suggest that nitrogen fixation may also enhance both MPn utilization and aerobic methane production (Fig. 3). An important feature of this new paradigm for methane production under P-stressed conditions is that it would scale on gross rather than net P assimilation because each round of coupled MPn production/decomposition would provide methane even if there were no net removal of P. This feature of the marine methane pump could result in a significantly enhanced flux of methane to the atmosphere compared with carbon dioxide sequestration that scales on net production (Fig. 3). Furthermore, a second potent greenhouse gas, nitrous oxide, may also be produced through coupled bacterial nitrification fuelled by excess ammonium production from nitrogen fixation. Nitrous oxide production by this mechanism would also scale on gross rather than net rates of nitrogen fixation because the gaseous by-product is not recycled, at least not on short timescales. The impact on the greenhouse-gas balance in this Fe-stimulated, nitrogen-fixation scenario, would likely be an enhancement, in part, due to the remineralization-intensive nature of the oceanic food webs (gross rates $\gg$ net rates) and also due to the greater greenhouse effects of nitrous oxide and methane compared with carbon dioxide. Continued greenhouse-gas-induced global warming could lead to enhanced stratification of the surface ocean and expansion of phosphate-limited, nitrogen-fixation-favourable marine habitats[33,36]. Such climate-driven modifications could reinforce aerobic methane production, resulting in accelerated greenhouse warming and the ecological consequences thereof.

FUTURE PROSPECTS

Research so far has focused on MPn decomposition; however, future studies focusing on MPn production pathways and their regulation will clarify *in situ* methane fluxes through the new pathway described herein. For example, is phosphonate production restricted to only a few species, or is it more commonplace? Does P limitation or climate variability affect the production of phosphonate compounds in the marine environment? The flux of methane from MPn metabolism in the marine environment is likely to be controlled by the supply of substrate, so it is essential that we identify the sources of phosphonates, especially MPn, and their role in the marine P cycle. Quantitative methods for the isolation and chemical characterization of dissolved and particulate P pools, including individual phosphonate compounds, are needed. A new method for concentrating low-molecular-weight dissolved organic matter, including phosphonates, holds great promise[37]. Hallmarks of microbial communities in nature are their enormous genomic diversity and complexity. Aerobic methane production is also likely to be more complex than we present here, and ecological predictions need to be tested under actual field conditions. We have recently established a centre to facilitate comprehensive studies in microbial oceanography, linking genomes to biomes, including ecosystem dynamics and climate modelling (cmore.soest.hawaii.edu). Hopefully we can make rapid progress on this and related challenges in the coming decade.

METHODS

SAMPLING

Surface seawater samples were collected at Station ALOHA (22°45′ N, 158° W) during monthly HOT research cruises using standard conductivity–temperature–depth rosette procedures. Ancillary environmental data, including dissolved oxygen, nutrients, particulate matter concentrations and rates of primary production, to name a few parameters, were collected as part of the HOT core measurement program (see http://hahana.soest.hawaii.edu/HOTDOGS). Sea water for MPn decomposition experiments was transferred from the 10 l polyvinylchloride sampling bottles into clean polycarbonate carboys before the start of each experiment. Nutrients (glucose, nitrate, phosphate or MPn) were added (see text) and subsamples were placed into 250 ml glass serum bottles that were capped with gas-tight Teflon-lined silicone stoppers and crimp-sealed with aluminium closures. A typical incubation treatment had 200 ml sea water and 50 ml headspace volume. The incubations were conducted at approximately *in situ* temperatures (23–26 °C) either under an approximately 12 h:12 h light–dark cycle or in total darkness depending on the experiment.

For field-collected *Trichodesmium* experiments, samples were collected in August 2007 during the C-MORE BloomER cruise by towing a 50-cm-mouth-diameter × 200-cm-length, 80-μm-mesh plankton net at a depth of 10–25 m at ~1 knot for a period of 10 min. The contents of the net were placed into a plastic beaker and diluted with 0.2 μm filtered surface sea water (FSW). *Trichodesmium* colonies, both fusiform and spherical, were identified and picked with a sterile plastic inoculation loop and placed into separate beakers containing FSW. Pooled samples of 10–20 colonies were then placed into individual 250 ml glass serum bottles fitted with Viton stoppers, spiked with MPn, phosphate or both, then incubated in an ondeck surface water temperature controlled incubator shaded to approximately 30% of ambient light for 4 days before methane analysis (see below). Uninoculated FSW with MPn added, and *Trichodesmium* colonies without MPn served as controls.

METHANE MEASUREMENTS

Methane determinations were made either by subsampling the headspace during a timed incubation, or by cryogenic gas extraction[3,5], depending on the anticipated methane concentration in the sample. Sample volumes ranged

from 10–500 µl and 100–150 ml for headspace and gas extraction, respectively. The methane was quantified using a gas chromatograph equipped with a flame ionization detector (Shimadzu, model GC-8A) following separation on a Porapak N column (3 mm internal diameter × 2 m length) using helium as the carrier gas. Calibration was made with a methane-in-nitrogen standard (100 p.p.m.; Matheson). Temperature settings were 60 °C for the column and 100 °C for both the injector and the detector. Calculations assumed a Bunsen coefficient of 0.024 for methane at 25 °C and a salinity of 34 p.p.t.

SCREENING OF MARINE METAGENOMIC LIBRARIES FOR PHOSPHONATE-UTILIZATION GENES

Two microbial community genomic data sets, one comprising 7 depths sampled at station ALOHA (ref. 18), and the other from the Sargasso Sea[19], were searched for known microbial phosphonate-utilization genes. A data set of C–P lyase genes from fully sequenced microbial genomes was used to search the ALOHA and Sargasso Sea gene libraries using tblastn with default parameters. The expectation score cutoff values used (*phnG*, 1×10^{-3}; *phnH*, 2×10^{-4}; *phnI*, 1×10^{-28}; *phnJ*, 2×10^{-60}; *phnK*, 5×10^{-40}; *phnI*, 4×10^{-31}; *phnM*, 2×10^{-32}) were those observed comparing orthologue genes within complete C–P lyase operons in marine bacteria (http://img.jgi.doe.gov). Pfam analyses were carried out to confirm the results. The final C–P lyase homologues recovered from the Sargasso Sea data were then summed, and normalized to the number of single-copy *recA* genes recovered from the same samples, as previously described[20].

ANCILLARY MEASUREMENTS

Dissolved organic carbon, nitrate and particulate C, N and P were measured using standard HOT program protocols; all methods and data are available at http://hahana.soest.hawaii.edu.

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Author contributions

D.M.K. formulated the hypothesis, directed the research and wrote the first draft of the manuscript. L.B. and K.B. carried out most of the experiments. E.F.D. and A.M. conducted and analysed the genome database search. All authors contributed to the experimental design, data interpretation and preparation of the final draft of the manuscript.

Author information

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