LETTERS
PUBLISHED ONLINE: 14 NOVEMBER 2016 | DOI: 10.1038/NGEO2837
nature geoscience

Marine methane paradox explained by bacterial degradation of dissolved organic matter

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Biogenic methane is widely thought to be a product of archaeal methanogenesis, an anaerobic process that is inhibited or outcompeted by the presence of oxygen and sulfate[1–3]. Yet a large fraction of marine methane delivered to the atmosphere is produced in high-sulfate, fully oxygenated surface waters that have methane concentrations above atmospheric equilibrium values, an unexplained phenomenon referred to as the marine methane paradox[4,5]. Here we use nuclear magnetic resonance spectroscopy to show that polysaccharide esters of three phosphonic acids are important constituents of dissolved organic matter in seawater from the North Pacific. In seawater and pure culture incubations, bacterial degradation of these dissolved organic matter phosphonates in the presence of oxygen releases methane, ethylene and propylene gas. Moreover, we found that in mutants of a methane-producing marine bacterium, *Pseudomonas stutzeri*, disrupted in the C–P lyase phosphonate degradation pathway, methanogenesis was also disabled, indicating that the C–P lyase pathway can catalyse methane production from marine dissolved organic matter. Finally, the carbon stable isotope ratio of methane emitted during our incubations agrees well with anomalous isotopic characteristics of seawater methane. We estimate that daily cycling of only about 0.25% of the organic matter phosphonate inventory would support the entire atmospheric methane flux at our study site. We conclude that aerobic bacterial degradation of phosphonate esters in dissolved organic matter may explain the marine methane paradox.

Nearly all proposed solutions to the methane paradox invoke some form of archaeal methanogenesis in micro-anaerobic environments or direct transfer of substrates between phytoplankton and attached archaeal epibionts[6–8]. Viable methanogens have been isolated from the gut contents of pelagic metazoans and from large, rapidly sinking particles, and archaea attached to phytoplankton cells have been visualized using fluorescent hybridization techniques. However, experimental measurements of oxygen penetration into organic flocs, aggregates, and sinking particles indicate that anaerobic conditions cannot persist for long on such very small scales, placing severe limits on the potential contribution of anaerobic methane production in microenvironments[9,10]. Likewise, it is not known if archaeal epibionts are methanogenic, or if methane is generated from archaeal–phytoplankton associations.

One novel and poorly studied pathway of aerobic methane production is the demethylation of methylphosphonate (MPn), a simple one-carbon, reduced phosphorus compound with a chemically stable C–P (phosphonate) bond[11,12]. Recognizing that microbial degradation of MPn might explain the methane paradox, Karl *et al.* showed that methane production could be simulated by amending seawater with exogenous MPn[13]. Evidence supporting Karl's aerobic methane hypothesis includes a recent report that showed MPn was present in *Nitrosopumilus maritimus*, a nitrifying Thaumarchaeota isolated from a marine aquarium[14], and that genes encoding the synthesis of phosphonopyruvate, a key intermediate in phosphonate biosynthesis, were common in a survey of ocean metagenomic data[15]. Nevertheless, ^{31}P nuclear magnetic resonance (NMR) spectra of suspended particulate organic matter collected from open ocean surface water were unable to detect the presence of phosphonates in microbial biomass[16,17], suggesting MPn synthesizing archaea are either not abundant in the euphotic zone[18,19], or do not accumulate significant amounts of this compound. Dissolved MPn itself has not been detected in seawater, and direct rate measurements of methane production from ^{14}C–MPn were too low to explain observed methane fluxes[20]. Microbial cycling of MPn is an attractive solution to the methane paradox since it provides a pathway for aerobic methanogenesis, but the environmental substrates and degradation pathways required to support Karl's proposed methylphosphonate-driven, aerobic methane hypothesis have not been demonstrated, so the relevance of this hypothesis to the marine methane cycle remains unclear.

Organic phosphorus compounds containing C–P bonds are notably abundant in 'semi-labile' dissolved organic matter (DOM), a large but poorly characterized reservoir of organic carbon, nitrogen and phosphorus that accumulates in surface waters of mid-ocean gyres[16,17,21]. The composition of DOM phosphonates has not been investigated, but we suspected that novel polysaccharides, which represent a large fraction of semi-labile DOM, might incorporate MPn esters[22]. Methane production in the upper ocean would then be a natural consequence of the seasonal to annual cycling of semi-labile DOM.

To chemically characterize DOM phosphonates, we collected semi-labile DOM at Station ALOHA, a long-term ecological study site located in the North Pacific subtropical gyre, and purified the polysaccharide fraction. We used ^{31}P NMR to characterize P functional groups, which exhibited major signals from phosphonate (Fig. 1; 20–31 ppm; ~21% total P), and phosphate diesters (−6 to +6 ppm ~73% total P), similar to previous P functional group analyses of semi-labile DOM using solid-state NMR techniques[18,21]. However, in our spectra, the phosphonate region is further distinguished by three broad peaks at 29.4 ppm, 24.9 ppm and 22.2 ppm, that appear in a 1:1.7:0.5 ratio, and by the presence of pyrophosphate (−12 to −6 ppm), which represents ~6% total P. ^{31}P–^{1}H heteronuclear multiple bond correlation (HMBC) experiments and high-resolution mass

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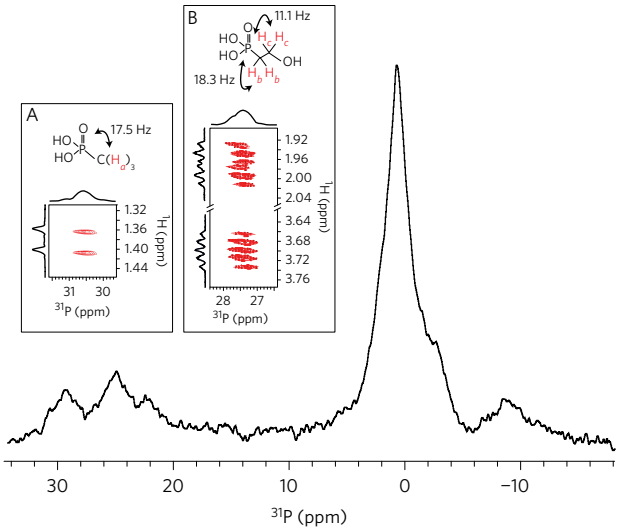

Figure 1 | Characterization of phosphorus in DOM by ^{31}P NMR spectroscopy. The ^{31}P NMR spectrum of DOM polysaccharides shows the presence of at least three phosphonates between 20–31 ppm, phosphate diesters (+6 to −6 ppm) and pyrophosphate (−6 to −12 ppm). Strong base hydrolyses phosphonate and phosphate esters, allowing us to use 2D (^{31}P, ^{1}H) HMBC experiments to identify phosphonates by chemical shift and splitting pattern. Using this approach, we assigned the downfield peak at 30.6 ppm as methylphosphonate (inset A), the peak at 27.5 ppm as 2-hydroxyethylphosphonate (inset B), and the upfield peak at 22.6 ppm as hydroxymethylphosphonate. Small differences in ^{31}P chemical shifts between the 1D and 2D spectra are due to changes in semi-labile DOM concentration and pH value between samples.

spectrometry of carbohydrate hydrolysis products allowed us to identify the phosphonate at 29.4 ppm as methylphosphonate (MPn, M + 1 = 97.0055; Supplementary Table 1; ^{31}P NMR (^{1}H decoupled, D_2O) δ: 29.4 ppm, ^{1}H δ: 1.38 ppm (d, J = 17.5 Hz) (authentic MPn, ^{31}P NMR δ: 29.4 ppm, ^{1}H δ: 1.38 ppm d, J = 17.8 Hz)), at 24.9 ppm as 2-hydroxyethylphosphonate (2-HEP; M + 1 = 127.0160; ^{31}P NMR (^{1}H decoupled, D_2O) δ: 24.9 ppm, ^{1}H δ: 3.75 ppm (dt, J = 11.7, 7.5 Hz), δ: 1.98 ppm (dt, J = 17.1 Hz, 7.4 Hz)) (authentic 2-HEP, ^{31}P δ: 24.9 ppm, ^{1}H δ: 3.75 ppm (dt, J = 11.1, 7.4 Hz), δ: 1.98 ppm (dt, J = 18.2, 7.5 Hz)) and at 22.2 ppm as hydroxymethylphosphonate (HMP; M + 1 = 113.0004; Supplementary Fig. 1). HMBC and mass spectra also revealed the presence of smaller amounts of 2-hydroxypropylphosphonate (2-HPP), phosphonoacetic acid, and phosphite (Supplementary Fig. 1).

Once we confirmed that semi-labile DOM includes polysaccharide-MPn esters, we proceeded to test if DOM degradation produces methane. When Station ALOHA seawater was amended with glucose and nitrate to relieve C and N nutrient limitation, only trace amounts of methane (~2.9 nM) and ethylene (0.6 nM) were produced. However, when DOM polysaccharide was added to the amendment, methane and ethylene were rapidly evolved (Fig. 2). In our first experiment, addition of semi-labile DOM increased methane to ~15 nM and ethylene to ~26 nM in less than 48 h. The increase in ethylene over controls (+25 nM) was approximately 2.1 times higher than the increase in methane (+12 nM), and similar to the ratio of 2-HEP: MPn in the added DOM (~1.7; Fig. 1). We also observed production of a small amount of propylene (0.4 nM) in the DOM-amended treatments from the degradation of 2-HPP. Subsequent experiments using DOM from other collection sites and periods likewise produced ethylene and methane in ratios (~1.6–1.8) similar to the 2-HEP:MPn ratio

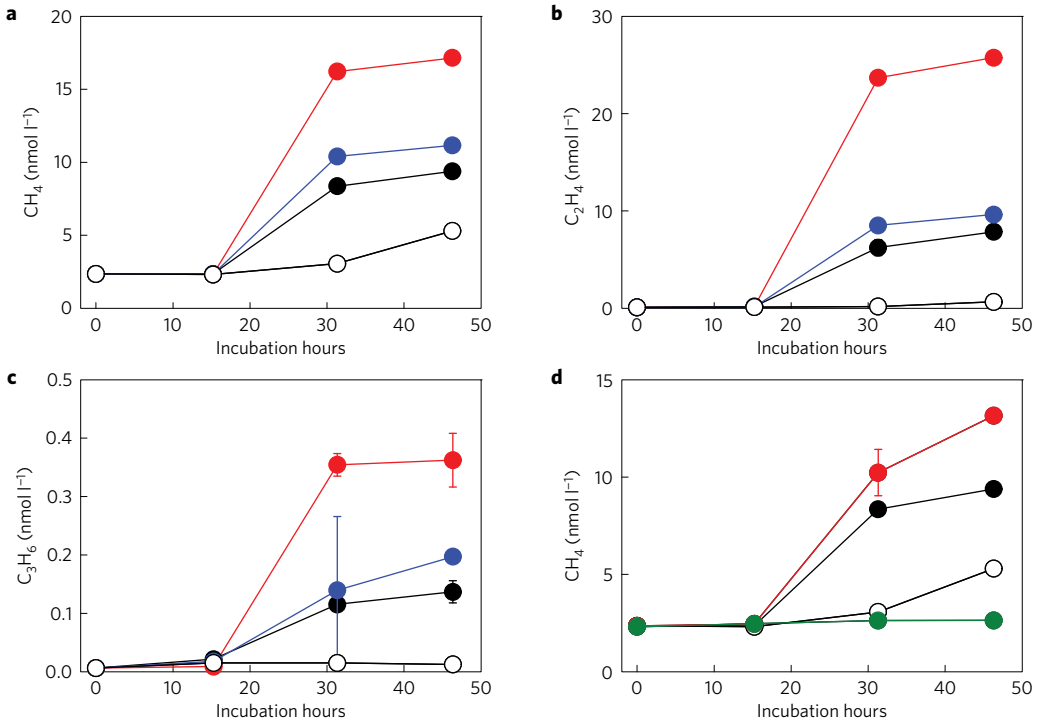

Figure 2 | Hydrocarbon gas production from semi-labile DOM. a–c, Net production of methane (**a**), ethylene (**b**) and propylene (**c**) from DOM phosphonates. Seawater amended with glucose and nitrate (C + N; white circles), and C + N + semi-labile DOM from Station ALOHA, 2007 (red circles), Station ALOHA, 2014 (black circles) and NELHA, 2015 (blue circles). **d**, Evolution of methane in treatments amended with C + N (white circles), and 48.6 mg l^{-1} (red circles) or 24.3 mg l^{-1} (black circles) Station ALOHA, 2014 semi-labile DOM. Phosphate addition (~1.5 μM) to the 48.6 mg l^{-1} treatment (C + N + P + semi-labile DOM; green circles) inhibited methane and ethylene (not shown) production. Error bars represent 1 s.d. of duplicate samples. When error bars are not visible, they are within the area of the symbol.

Table 1 | Net production of methane and ethylene from methylphosphonate and semi-labile DOM by Station ALOHA wild type and mutant (*phnK*491::Tn*5*) *P. stutzeri*.

Sample	Methane (nM)		Ethylene (nM)	
	Wild type	*phnK*491::Tn*5*	Wild type	*phnK*491::Tn*5*
Control (C + N)	0.45 ± 0.11	0.07 ± 0.07	0.09 ± 0.00	0.07 ± 0.02
Semi-labile DOM	10.28 ± 0.56	0.04 ± 0.07	17.26 ± 0.38	0.07 ± 0.06
Methylphosphonate	447.79	0.03 ± 0.03	0.13	0.05 ± 0.04

The wild type *P. stutzeri* with the C–P lyase pathway readily degrades methylphosphonate to methane, while the *phnK* mutant strain does not. Likewise, the wild type *P. stutzeri* produces methane and ethylene from semi-labile DOM, while the *phnK* mutant strain does not. All treatments were amended with glucose and ammonium. Errors indicate the 1 s.d. of duplicate sample measurements. Measurements with no errors consisted of single samples.

in these DOM samples (~1.2; Fig. 2). In two sets of parallel experiments, we found that oxygen decreased over the course of the 48 h incubation relative to controls with no semi-labile DOM amendment, but concentrations were always >140 μM O_2, confirming hydrocarbon gas generation under oxic conditions (Supplementary Fig. 2).

Diverse bacterial lineages encode the C–P lyase pathway, a multi-enzyme complex that mediates the degradation of alkylphosphonates[23]. A dilution-to-extinction cultivation approach using Station ALOHA seawater enriched with DOM polysaccharides allowed us to isolate a number of DOM-responsive bacterial strains, including a phosphonate-degrading *Pseudomonas stutzeri* strain (HI00D01), capable of growing on semi-labile DOM as the sole source of phosphorus. When grown in inorganic phosphate-depleted medium, *P. stutzeri* HI00D01 rapidly released methane and ethylene from DOM polysaccharides. However, a mutant (*phnK*491::Tn*5*) of this same *P. stutzeri* strain, that was disrupted in the *phnK* gene (which encodes a protein required for phosphonate degradation), was completely disabled in methane and ethylene production (Table 1).

Water column concentrations of methane increase with depth to a shallow, subsurface maximum (~200–300 m at Station ALOHA), followed by a slow decrease to depths >1,500 m (refs 4,24). Nearly all methane produced above the subsurface maximum escapes to the atmosphere. Carbon stable isotope ($\delta^{13}C$) values of the methane inventory within the ventilated upper water column range from −44‰ to −47‰ (refs 24,25), and are more isotopically enriched than methane typically associated with anaerobic methanogenesis (−50 to −110‰). These unusual isotopic values of open ocean methane indicate that unique, aerobic methane-forming processes occur in the upper water column. Air–sea gas exchange is accompanied by a small isotopic fractionation that delivers slightly enriched methane of ~−42‰ to the atmosphere[24]. A source of methane with a $\delta^{13}C$ ratio close to the value of ventilated methane is therefore needed to maintain steady-state isotopic balance. Methane recovered from our DOM-amended treatments had a $\delta^{13}C$ value of −39‰, very similar to the value of ventilated methane.

Methane flux to the atmosphere at Station ALOHA ranges between 1.6 and 2.5 μmoles m^{-2} d^{-1}, and are typical of fluxes measured in mid-ocean gyres. Daily cycling of only 0.2–0.3% of the MPn inventory would support the entire atmospheric methane flux at this site. A flux of this magnitude implies a residence time of DOM polysaccharide of 1–2 yr, which is in good agreement with residence time estimates made from natural abundance radiocarbon measurements (1–3 yr)[26]. Likewise, if MPn is indeed the principal source of upper ocean methane, we calculate a total phosphonate synthesis rate ((methane flux × total phosphonate)/MPn) in the upper 300 m of the water column of 3.5–8.3 μmoles m^{-2} d^{-1}, or about 0.5–3% of the 250–750 μmoles m^{-2} d^{-1} euphotic zone P demand[27]. This estimate agrees well with a recent estimate of phosphorus redox cycling using radiotracer (^{33}P) methods, which found reduced P (III) species (phosphite and phosphonates) represent 1–3% of microbial phosphate uptake in surface waters of the Sargasso Sea[28].

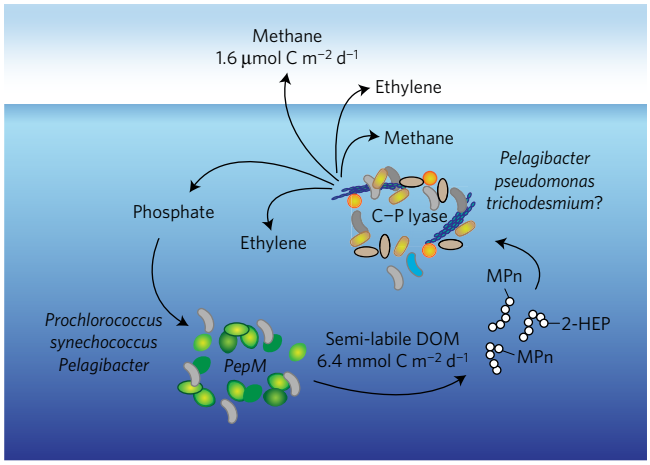

Figure 3 | Production of methane and ethylene from semi-labile DOM phosphonates. Metagenomic data suggests *Prochlorococcus*, a cyanobacteria that contributes approximately half of ocean primary production, can synthesize phosphoenolpyruvate mutase (*PepM*), a key enzyme for phosphonate synthesis. *Prochlorococcus* is probably a major source of semi-labile DOM phosphonates. Bacteria with C–P lyase degrade semi-labile DOM polysaccharides and release methane and ethylene. Most methane in surface water is lost to the atmosphere, while most ethylene is consumed by heterotrophic bacteria. From the flux of methane, we estimate ~10% of primary production is used to synthesize semi-labile DOM polysaccharides which have a residence time of ~1–2 years.

Our study links the ocean-to-atmosphere flux of methane, a potent greenhouse gas, to the degradation of semi-labile DOM (Fig. 3). Methane production from semi-labile DOM phosphonates may also explain methane distributions and isotope values in oligotrophic lakes[29]. Freshwaters accumulate semi-labile DOM polysaccharides that are chemically indistinguishable from marine DOM, and it is highly likely that these polysaccharides also incorporate MPn esters. Phosphonate synthesis gene homologues are present in *Prochlorococcus* and *Pelagibacter*[15], the dominant photoautotrophic and heterotrophic bacterial groups found in oligotrophic gyres, and these bacteria are probably major contributors to semi-labile DOM. Phosphate can be regenerated from semi-labile DOM phosphonates through the C–P lyase pathway, explaining the broad distribution of this pathway among marine bacteria revealed by ocean metagenomic surveys[30]. Methane and DOM phosphonates are present throughout the water column, and low rates of MPn metabolism, even in the presence of high phosphate concentrations, may be an unrecognized source of methane to the deep sea. Our new results show that daily cycling of only a small fraction of semi-labile DOM polysaccharide-MPn esters readily resolves the longstanding 'marine methane paradox' (Fig. 3).

Methods

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of this paper.

Received 11 July 2016; accepted 13 October 2016; published online 14 November 2016

References

1. Thauer, R. K., Kaster, A.-K., Seedorf, H., Buckel, W. & Reiner, H. Methanogenic archaea: ecologically relevant differences in energy conservation. *Nat. Rev. Microbiol.* **6,** 579–591 (2008).
2. Garcia, J.-L., Patel, B. K. & Ollivier, B. Taxonomic, phylogenetic and ecological diversity of methanogenic archaea. *Anaerobe* **6,** 205–226 (2000).
3. Liu, Y. & Whitman, W. B. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Ann. NY Acad. Sci.* **1125,** 171–189 (2000).
4. Reeburgh, W. S. Oceanic methane biogeochemistry. *Chem. Rev.* **107,** 486–513 (2007).
5. Kiene, R. P. *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethanes* (ASM, 1991).
6. Oremland, R. S. Methanogenic activity in plankton samples and fish intestines: a mechanism for *in situ* methanogenesis in oceanic surface waters. *Limnol. Oceanogr.* **24,** 1136–1141 (1979).
7. De Angelis, M. A. & Lee, C. Methane production during zooplankton grazing on marine phytoplankton. *Limnol. Oceanogr.* **39,** 1298–1308 (1994).
8. Ditchfield, A. K. *et al*. Identification of putative methylotrophic and hydrogenotrophic methanogens within sedimenting material and copepod faecal pellets. *Aquat. Microbiol. Ecol.* **67,** 151–160 (2012).
9. Ploug, H., Kuhl, M., Buchholz-Cleven, B. & Jorgensen, B. B. Anoxic aggregates-an ephemeral phenomenon in the pelagic environment? *Aquat. Microbiol. Ecol.* **13,** 285–294 (1997).
10. Simon, M., Grossart, H., Schweitzer, B. & Ploug, H. Microbial ecology of organic aggregates in aquatic ecosystems. *Aquat. Microbiol. Ecol.* **28,** 175–211 (2002).
11. Cook, A. M., Daughton, C. G. & Alexander, M. Phosphonate utilization by bacteria. *J. Bacteriol.* **133,** 85–90 (1978).
12. Kamat, S. S., Williams, H. J., Dangott, L. J., Chakrabarti, M. & Raushel, F. M. The catalytic mechanism for aerobic formation of methane by bacteria. *Nature* **497,** 132–136 (2013).
13. Karl, D. M. *et al*. Aerobic production of methane in the sea. *Nat. Geosci.* **1,** 473–478 (2008).
14. Metcalf, W. W. *et al*. Synthesis of methylphosphonic acid by marine microbes: a source for methane in the aerobic ocean. *Science* **337,** 1104–1107 (2012).
15. Yu, X. *et al*. Diversity and abundance of phosphonate biosynthetic genes in nature. *Proc. Natl Acad. Sci. USA* **110,** 20759–20764 (2013).
16. Kolowith, L. C., Ingall, E. D. & Benner, R. Composition and cycling of marine organic phosphorus. *Limnol. Oceanogr.* **46,** 309–320 (2001).
17. Sannigrahi, P., Ingall, E. D. & Benner, R. Cycling of dissolved and particulate organic matter at station ALOHA: insights from ^{13}C NMR spectroscopy coupled with elemental, isotopic and molecular analyses. *Deep-Sea Res. I* **52,** 1429–1444 (2005).
18. Karner, M. B., DeLong, E. F. & Karl, D. M. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature* **409,** 507–510 (2001).
19. Santoro, A. E. *et al*. Genomic and proteomic characterization of "*Candidatus Nitrosopelagicus brevis*": an ammonia-oxidizing archaeon from the open ocean. *Proc. Natl Acad. Sci. USA* **112,** 1173–1178 (2015).
20. del Valle, D. & Karl, D. M. Aerobic production of methane from dissolved water column methylphosphonate and sinking particles in the North Pacific Subtropical Gyre. *Aquat. Microbiol. Ecol.* **73,** 93–105 (2014).
21. Young, C. L. & Ingall, E. D. Marine dissolved organic phosphorus composition: insights from samples recovered using combined electrodialysis/reverse osmosis. *Aquat. Geochem.* **16,** 563–574 (2010).
22. Aluwihare, L. I., Repeta, D. J. & Chen, R. F. A major biopolymeric component to dissolved organic carbon in surface sea water. *Nature* **387,** 166–169 (1997).
23. McGrath, J. W., Chin, J. P. & Quinn, J. P. Organophosphonates revealed: new insights into the microbial metabolism of ancient molecules. *Nat. Rev. Microbiol.* **11,** 412–419 (2013).
24. Holmes, E. M., Sansone, J. F., Rust, T. M. & Popp, B. N. Methane production, consumption, and air-sea exchange in the open ocean: an evaluation based on carbon isotopic ratios. *Glob. Biogeochem. Cycles* **14,** 1–10 (2000).
25. Sasakawa, M. *et al*. Carbon isotopic characterization for the origin of excess methane in subsurface seawater. *J. Geophys. Res.* **113,** C03012 (2008).
26. Repeta, D. J. & Aluwihare, L. I. Radiocarbon analysis of neutral sugars in high molecular weight dissolved organic carbon: implications for organic carbon cycling. *Limnol. Oceanogr.* **51,** 1045–1053 (2006).
27. Björkman, K. & Karl, D. M. Bioavailability of dissolved organic phosphorus in the euphotic zone at Station ALOHA, North Pacific Subtropical Gyre. *Limnol. Oceanogr.* **48,** 1049–1057 (2003).
28. van Mooy, B. A. S. *et al*. Major role of planktonic phosphate reduction in the marine phosphorus redox cycle. *Science* **348,** 783–785 (2015).
29. Bogard, M. J. *et al*. Oxic water column methanogenesis as a major component of aquatic CH_4 fluxes. *Nat. Commun.* **5,** 5350 (2014).
30. Martinez, A., Tyson, G. W. & Delong, E. F. Widespread known and novel phosphonate utilization pathways in marine bacteria revealed by functional screening and metagenomic analyses. *Environ. Microbiol.* **12,** 222–238 (2010).

Acknowledgements

We thank the Captain and crew of the RV *Kilo Moana* as well as K. Olson and the staff at the Natural Energy Laboratory, Hawaii Authority for assistance in sample collection. We also thank R. Boiteau for his assistance with mass spectrometry, A. Bate for helping with gas chromatography measurements, K. Doggett for flow cytometry analysis and the Hawaii Ocean Time-series team for facilitating part of this work. This research is a contribution of the Daniel K. Inouye Center for Microbial Oceanography: Research and Education (C-MORE) and was supported by the Gordon and Betty Moore Foundation (through Grants GBMF3298 to D.J.R. and E.F.D., GBMF3777 to E.F.D. and GBMF3794 to D.M.K.), as well as a grant from the Simons Foundation (SCOPE Award ID 329108 to D.J.R., E.F.D. and D.M.K.).

Author contributions

D.M.K., D.J.R. and E.F.D. designed the experiments with input from all co-authors. D.J.R. collected and purified DOM samples, and performed spectral analyses. S.F. performed microcosm incubations and trace gas analyses with input from D.M.K. O.A.S. isolated DOM degrading microbes, performed genomic analyses, and performed microcosm incubations and trace gas analyses with input from E.F.D. C.G.J. performed NMR spectral analyses of DOM. L.D.R. performed chemical degradation experiments and identified phosphonates in DOM. M.A. purified DOM and assisted in microcosm incubation experiments. D.J.R. drafted the manuscript with input from all authors.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to D.J.R.

Competing financial interests

The authors declare no competing financial interests.

Methods

Semi-labile DOM isolation. Station ALOHA seawater (22° 45′ N, 158° 00′ W) was drawn from 15 m using a deck-mounted diaphragm pump, or directly from the 20 m seawater tap at the Natural Energy Laboratory, Hawaii Authority (NELHA) facility. Samples were filtered in-line and the high molecular weight fraction (<0.2 μm) concentrated using a cross-flow ultrafiltration system fitted with a GE-osmonics GE series membrane. The membrane has a pore size of ∼1 nm and nominally retains organic matter of molecular weight >1 kDa (>99% rejection of vitamin B_{12} in laboratory tests). Approximately 1,800 l of seawater was concentrated daily to 20 l, frozen and returned to shore for further processing.

In the lab, concentrates were thawed and filtered through an Ultracel 30 kD membrane (Millipore) to remove viruses and other small particles, reduced in volume to 2 l by ultrafiltration, and desalted by serial (10×; 2 l each) dilution/concentration with ultrahigh-purity water. Samples from different concentrates were pooled, and freeze-dried to a fluffy white powder that was ∼35–38% carbon. Between 18 and 21% of seawater total dissolved organic carbon was recovered. We did not collect blanks of the ultrafiltration/desalting system for these particular experiments. However, measurements from past sampling efforts showed that carbon blanks were typically <0.0003% of sample carbon. No carbohydrate was detected in the system blank by ^{1}HNMR spectroscopy. Blanks for carbohydrate purification protocols were estimated to be <0.05% of total carbon and, like the system blank, did not contain any detectable carbohydrate.

The polysaccharide fraction was purified in 0.5 g batches by dissolving freeze-dried organic matter in 5 ml of water, and applying this solution to a 2 cm inner diameter column packed with 16 g anion exchange resin (BioRad 5, 100–200 mesh) in hydroxide form. Carbohydrates were washed from the column with 80 ml water, followed by 80 ml of 1 M formic acid. Cations were removed by stirring the wash for 4 h over 1 g AG 50W-X8 resin (hydrogen form, BioRad). The sample was freeze-dried, and traces of formic acid removed by repeated freeze-drying (3×) from pH 3 high-purity water. Natural abundance radiocarbon measurements of the purified polysaccharide fraction performed at the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility in Woods Hole yielded Δ^{14}C values between +55 to +59‰ , similar to dissolved inorganic carbon (+40‰), indicating this fraction is comprised of semi-labile DOM.

Chemical and spectroscopic analyses. For carbon and nitrogen elemental and isotope analyses, samples (typically 4–8 mg) were weighed into tin capsules (9 × 10 mm; Costech), which were carefully folded and submitted for analysis to the University of California, Davis, Stable Isotope Facility (UCDSIF; http://stableisotopefacility.ucdavis.edu). A description of the instruments and handling protocols can be found at: http://stableisotopefacility.ucdavis.edu/13cand15n.html. Each sample was run in triplicate with numerous sample blanks (folded tin capsules with no sample inside) interspersed throughout the analysis. In all cases, carbon and nitrogen in blanks were below the limit of detection. Phosphorus was determined by wet chemical oxidation, followed by colorimetric (molybdenum blue) assay[31]. Our samples yielded C/N/P ratios of ∼300:21:1, with δ^{13}C values of ∼−22‰ and δ^{15}N of ∼+6‰.

NMR spectra were acquired at 25 °C in 20% or 100% D_2O on a Bruker 400 AVANCE spectrometer 400-DPX (400 MHz for ^{1}H, and 162 MHz for ^{31}P) fitted with a 5 mm inverse broadband probe and running TOPSPIN 1.3. Proton shifts are reported relative to water at 4.7 ppm. Phosphorus shifts are relative to external 85% phosphoric acid at 0 ppm. For proton-decoupled ^{31}P spectra, we used 'zgdc30' with WALTZ16 decoupling and a 10 s relaxation delay, 121 K scans and 30 Hz line broadening. ^{1}H NMR spectra are in agreement with previous analyses of DOM polysaccharides sampled by ultrafiltration (Supplementary Fig. 3a), with major signals at 4.5–5.5 ppm (anomeric H; $(O)_2C\mathbf{H}$), 3.5–4.5 ppm (O-alkyl; HO-C**H**), 2.7 ppm (methyl amino sugar; $C\mathbf{H}_3N$-), 2.0 ppm (acetamide; N-CO-CH_3), and at 1.3 ppm (deoxysugar; HOC-$C\mathbf{H}_3$)[32].

Although anion exchange chromatography efficiently removes nucleic acids and proteins from the sample, given the high C/P ratio of the final products, trace quantities of these biochemicals, particularly nucleic acids, could contribute small amounts of phosphorus that would be difficult to detect by NMR. Therefore, we estimated the maximum contribution of nucleic acids (NA) spectrophotometrically at 260 nm, by assuming all the adsorption at this wavelength was from NA, and using the equation NA(mg ml^{-1}) = 40(A)/[DOC], where A = adsorption at 260 nm, [DOC] is the concentration of sample carbon per ml, and 40 is the conversion factor (mg ml^{-1}). We further assumed that nucleic acids are 10% by weight phosphorus. Using this approach, we calculate that >85% of the phosphorus in our samples is associated with carbohydrate. Most likely, this estimate is conservative, as small amounts of humic substances, which absorb to wavelengths >300 nm, are known to co-elute with our polysaccharide fraction. Additionally, adsorption spectra of our samples appear as a smooth decrease in absorbance with increasing wavelength, again characteristic of humic substances (Supplementary Fig. 3b).

Heteronuclear multiple bond correlation (HMBC) experiments were performed after treating a portion of the sample (3–5 mg) with 1 ml of 2N aqueous KOH, 100 °C for 96 h. The product was stirred (8 h) over AG 50W-X8 resin to remove potassium, dried, and dissolved in 100% D_2O. In ^{31}P NMR spectra, phosphonates appear as sharp peaks atop broader peaks, indicating the sample was not fully hydrolysed. Gradient-enhanced HMBC ('hmbcgplpndqf') spectra were optimized at 7.5 Hz long-range couplings, with a low-pass J filter, no decoupling during acquisition, 3.5 s relaxation delay, 1,000 μs gradient ratios of Z1 = Z2 = 12.34%, and Z3 = 10%. Real spectrum size was 4,096 × 512, 64 scans per FID (free induction decay). FID processing used a Gaussian broadening maximum of 0.1 and line broadening of −5.0 Hz.

High-resolution mass spectra were acquired on a dried, hydrolysed sample that had been extracted with acidic methanol. The methanol extract was dried under vacuum and the sample dissolved in 3M aqueous formic acid. Phosphonates were isolated by ion exchange chromatography (Hamilton RCX-30, 4.6 × 250 mm, 7 μm; eluted at 1 ml min^{-1} with 3M formic acid) and refractive index detection. The 6–7 min fraction was dried, dissolved in 50/50 methanol/0.1% aqueous formic acid, and infused into a Thermo-Fisher Fusion orbitrap spectrometer equipped with a heated electrospray ionization source (ESI) at 3 μl min^{-1}. ESI source parameters were set to a capillary voltage of 3,500 V, sheath and auxiliary gas flow rates of 5 and 3 (a.u.), and ion transfer tube and vaporizer temperatures of 275 °C and 50 °C. Mass scans were collected in high-resolution (500 K) positive mode. Phthalic anhydride (m/z 149.02383) was used as an internal calibrant. A list of major ions, elemental formulae, and preliminary assignments are provided in Supplementary Table 1.

Microcosms and hydrocarbon gas analyses. Unfiltered seawater was collected by Niskin bottles mounted to a rosette. Seawater for experiment 1 was collected from 25 m at Station ALOHA in November 2015 (Hawaii Ocean Time-series cruise 278). Water for experiment 2 was collected from 15 m in August 2015 at a site north of Station ALOHA in the North Pacific subtropical gyre at 22° 45′ N, 158° 00′ W. Upon recovery, water was transferred to a 20 l polycarbonate carboy. The carboy was amended with glucose and nitrate (C + N) to a final concentration of ∼100 μM C and ∼16 μM N, respectively. Samples were siphoned into 72 ml glass serum bottles with experimental treatments further amended with either 100 or 200 μl of a semi-labile DOM solution (17.5 mg ml^{-1}). Afterwards, phosphate was added to the carboy to a final concentration of ∼1.5 μM (C + N + P), and additional samples were collected and amended with 100 or 200 μl of the semi-labile DOM solutions. All samples were sealed with Teflon-lined butyl rubber stoppers, crimp-sealed, and incubated in a dark flow-through incubator maintained at near *in situ* temperature. At every time point (15, 31, and 46 h), duplicate samples from each treatment were fixed with saturated mercuric chloride solution (∼0.1% of the total sample volume) to inhibit biological activity. In addition, duplicate samples were collected from the main carboy before and after phosphate addition, and fixed with mercuric chloride to measure the initial concentrations of dissolved gases.

Similarly, triplicate samples for the determination of methane isotopic value were amended with glucose (∼150 μM C), nitrate (∼24 μM N), and semi-labile DOM from NELHA 2015 (∼50 mg l^{-1}) in 160 ml serum bottles, and incubated for 48 h in a dark flow-through incubator. After incubation, the samples were preserved by addition of hydrochloric acid to pH < 2, and sent to UCDSIF. Before analysis, a 30 ml helium headspace was created in each bottle, and samples were allowed to reach equilibrium. Methane carbon isotope values were measured using a ThermoFisher PreCon system interfaced to a Delta V Plus stable isotope mass spectrometer. Details of the analyses can be found at: http://stableisotopefacility.ucdavis.edu/ch4.html.

Dissolved methane, ethylene and propylene concentrations were measured by gas chromatography using a gas stripping and cryo-trap concentration method[33]. The sample was weighed and loaded into a sparging chamber, where the dissolved gases were extracted by bubbling with ultrahigh-purity (UHP) helium. During the gas stripping procedure, the gas stream passed through Drierite and Ascarite columns to remove water vapour and carbon dioxide, and the gases were concentrated in a 80–100 mesh Porapak Q trap cooled in liquid N_2. Once the gas extraction was completed (12 min), the trap was heated for 2 min with boiling water (∼90–100 °C) and the concentrated gases released and injected into the gas chromatograph (Agilent 7980A) using UHP helium as carrier gas. The gases were separated on a GS-CarbonPLOT capillary column (30 m × 0.32 mm × 1.5 μm) and analysed with an FID at 450 °C. For the first minute of the run, the oven temperature was maintained at 40 °C, and then programmed to 65 °C at 75 °C min^{-1}, held for 1.3 min, increased again at 75 °C min^{-1} to 150 °C, then held for 2.2 min. The FID was calibrated by injecting different sized loops of a gas mixture standard containing 10 ppm of methane, ethylene and propylene in pure N_2 (Scott-Marrin). The loops were injected into the purging chamber and concentrated into the Porapak Q trap, following the same procedure as for the water samples.

Subsamples (1 ml) were transferred to a 2 ml cryovial, fixed with 32 μl of 16% paraformaldehyde, allowed to rest in the dark at room temperature for 15 min, then flash frozen in liquid nitrogen and stored at −80 °C for flow cytometry (FCM) analyses. FCM samples were thawed and stained with SYBR green I (Invitrogen, 0.01% v/v final concentration), and SYBR-stained cells were counted using an Influx Mariner flow cytometer.

Dissolved oxygen concentrations during the incubations were determined using the oxygen to argon molar ratios measured by membrane inlet mass spectrometry (MIMS)[33]. We used a HiQuad quadruple mass spectrometer with a cross-beam ion source and a Faraday collector, connected to a membrane inlet[34,35]. Briefly, water is pumped at a constant rate of ~2 ml min^{-1} through temperature-controlled capillary stainless steel tubing and through a 2.5-cm-long semipermeable microbore silicone membrane (Silastic, DuPont) located inside the inlet vacuum line. A fraction of the dissolved gases are transferred by vacuum through a liquid nitrogen trap to remove water vapour and carbon dioxide, before entering the mass spectrometer. For this study, masses 32 and 40 were recorded. To calibrate and account for instrument drift during analyses, a standard consisting of 0.2 μm filtered seawater air-equilibrated to 23°C (±0.01°C) was run every six samples (~30 min). Dissolved oxygen and argon concentrations in the standard were calculated using the solubility equations[36,37]. The typical precision of O_2/Ar ratios from triplicate samples is ±0.05%, whereas that of the dissolved gas is typically ±0.4%. At any time point, the dissolved oxygen concentration, $[O_2]_t$, was determined as:

$$[O_2]_t = \frac{(O_2/Ar)_t}{(O_2/Ar)_i} \times [O_2]_i \quad (1)$$

where the subscript *i* refers to the initial time zero sample.

Isolation and characterization of *Pseudomonas stutzeri* HI00D01. *P. stutzeri* HI00D01 was isolated from DOM-enriched dilution-to-extinction cultures prepared with seawater collected at 200 m depth in Station ALOHA during the Hawaii Ocean Time-series cruise 250 on 9 March 2013 following the methodology described by Sosa and colleagues[38]. Isolate HI00D01 was identified by whole genome shotgun (WGS) sequencing and a draft genome assembly is available under NCBI Bioproject PRJNA305749. SSU rRNA gene sequence analysis placed HI00D01 in the genus *Pseudomonas* sensu stricto, which includes several strains isolated from the marine environment such as *P. balearica* and *P. xanthomarina*[39]. Single colonies of HI00D01 were purified in marine agar (DIFCO 2216, BD) by sequential streaking. Colonies were wrinkled in appearance and tan in coloration, with a hard, dry surface, although a few smooth colony types were observed after streaking. Partial SSU rRNA gene sequences (1357-1369 nucleotide length) obtained by PCR amplification and Sanger sequencing (BigDye v3.1 protocol; Applied Biosystems) of a wrinkled colony type (sub-strain RA1) and four smooth colonies purified on marine agar were identical to the full-length sequence (1533 nucleotide length) assembled with WGS reads, except for two smooth colonies with the same deletion in position 209.

Evaluation of *P. stutzeri* HI00D01 growth on alkylphosphonates as the sole source of phosphorus. *P. stutzeri* HI00D01 was able to utilize several alkylphosphonates as sole phosphorus source in agarose media containing glucose (Supplementary Fig. 4). Test media was prepared with MOPS minimal media supplied without inorganic phosphate (M2101 Teknova; Hollister) in ultrapure water and 1.7% SeaKem LE agarose (Lonza), a high-purity agarose to minimize background growth. Glucose (0.2%) and phosphonates (final concentration of 100 mM) were added after autoclaving MOPS agarose media. Test plates were spotted with 10 μl culture samples and incubated in darkness at 26 °C for 5 days. HI00D01 tested positive for growth on methyl phosphonate (98%; Sigma-Aldrich 289868; St. Louis), ethylphosphonate (98%; Sigma-Aldrich 289876), (2-amino)ethylphosphonate (99%; Sigma-Aldrich 268674), 2-phosphonopropionate (98%; VWR AAAL11588-04; Radnor), 3-phosphonopropionate (94%; Sigma-Aldrich 228559), and (3-amino)propylphosphonate (99%; Sigma-Aldrich 268615) and negative on aminomethylphosphonate (98%; Sigma-Aldrich 324817) and 2-phosphonobutyric acid (97%; VWR AAAL12036-04). The lack of growth on aminomethylphosphonate is consistent with the absence of *phnO* in the *P. stutzeri* operon[40], which in *Escherichia coli* is required for catabolism and detoxification of (1-amino)alkylphosphonic acids[41,42]. On the other hand, 2-phosphonobutyric acid may fall outside the substrate range of the C–P lyase or phosphonate transporters, and thus cannot be used as a phosphorus source.

Transposon mutagenesis of *P. stutzeri* HI00D01 and identification of strains unable to metabolize phosphonates. Single colonies of HI00D01 derived from sub-strain RA1 were grown in 5 ml of marine broth (DIFCO 2216, BD) and incubated at 30 °C and 255 r.p.m. overnight. Cells were harvested by centrifugation at 2,000*g* for 5 min; the supernatant was discarded, and the pellet was washed with 10 ml of chilled 10% glycerol solution. Centrifugation and wash steps were repeated, after which cells were concentrated in 100 μl of 10% glycerol. The cell suspension was transformed with 40 ng of EZ-Tn5 ⟨KAN-2⟩ Tnp Transposome (Epicentre Biotechnologies) by electroporation in a 2 mm cuvette (12.5 kV cm^{-1}, 200 Ω, and 25 μF). To facilitate cell outgrowth and expression of the kanamycin antibiotic marker, immediately after electroporation, 0.4 ml of marine broth was added to the cuvette to dilute cells and incubated at 30 °C for 30 min. 50 μl aliquots of the dilute cell suspension were plated on large Petri dishes with marine agar media containing kanamycin (50 μg ml^{-1}) to select for transposon insertion mutants. Plates were incubated at 30 °C overnight. A robot Q-pix Genetix (Molecular Devices) was used to pick colonies from selective media to inoculate a total of sixteen 96-well microtiter plates containing marine broth and kanamycin. Plates were incubated at 30 °C for 2 days and cryopreserved with 20% glycerol.

To identify strains that could no longer use phosphonates, the Tn5 transposon mutant library was screened in Hawaii seawater agarose medium (1.7% SeaKem LE agarose) supplemented with 2-aminoethyl phosphonate (99% purity; Sigma-Aldrich 268674) as the sole phosphorus source and 0.2% glucose. The medium was amended with sodium nitrate (1 mM), ammonium chloride (1 mM), and kanamycin (50 μg ml^{-1}). To assess mutant viability, the library was screened in parallel on marine agar plates with kanamycin (50 μg ml^{-1}). Only 3 of 1,344 mutants screened failed to grow on marine agar. A total of 28 viable mutants were identified that failed to grow or grew poorly on the phosphonate screening media. Of these 28 mutants, 12 could form colonies on agarose media amended with glucose and inorganic phosphate as the sole phosphorus source, indicating that the rest carried mutations that rendered them auxotrophic for other nutrients. Further testing identified 3 mutants that could clearly grow on agarose medium with glucose and inorganic phosphate but failed to metabolize 2-aminoethyl phosphonate (Supplementary Fig. 4). To characterize the mutation in these strains, genomic DNA was purified and sequenced as described previously[38]. BLASTn analysis revealed that two of the strains had transposon insertions in *phnK* and the other strain had an insertion in *phnM*, both genes required for phosphonate utilization[42]. Mutations in additional C–P lyase pathway genes were not identified, most likely because of the small size of the library screened (1,344 mutants compared to the draft genome's ~4,600 predicted CDSs). Further testing confirmed that one of the *phnK* mutants (strain *phnK*491::Tn*5*) failed to utilize either of the phosphonate compounds for which the wild type strain tested positive for growth.

Aerobic production of hydrocarbon gases in *P. stutzeri* HI00D01 batch cultures amended with phosphonates or semi-labile DOM polysaccharides. Batch culture incubations of *P. stutzeri* HI00D01 grown in the presence of phosphonates were expected to accumulate hydrocarbon gases produced via the C–P lyase degradation pathway. To induce utilization of phosphonates, wild type and *phnK*491::Tn*5* cultures were pre-grown in 0.85 l of phosphate-free MOPS medium supplemented with 100 μM carbon (glucose). The mutant strain media contained kanamycin (50 μg ml^{-1}) to ensure selection of the transposon insertion. Wild type and mutant cultures in stationary phase were stimulated with 1 μM and 10 μM phosphate, 100 μM carbon (glucose), or a combination of phosphate and glucose, to determine if phosphorus had become limiting. Cultures only continued to grow when 10 μM phosphate and glucose were replenished, and were thus deemed phosphorus-starved. Once such conditions were established, the 0.85 l cultures were brought up to 1 l and amended with 100 μM carbon (glucose). In addition, samples were bubbled with filtered air to saturate oxygen. To prepare batch incubations with phosphonates, triplicate culture samples were aliquoted into 40 ml gas-tight glass bottles sealed with Teflon-lined butyl stoppers and no headspace. Treatments consisted of culture samples amended with methyl phosphonate (1 μM) or semi-labile DOM polysaccharides (95 μg ml^{-1}). The control treatment consisted of triplicate culture samples with no additional phosphorus. Samples were incubated for 1.75 days at 26 °C under a diurnal 12 h light/dark cycle.

Two additional samples of wild type and mutant *phnK*491::Tn*5* cultures were aliquoted into 40 ml gas-tight glass bottles and killed with 50 μl of saturated mercuric chloride solution to determine initial methane and ethylene concentrations. At the end of the incubation, two samples from each treatment were likewise killed with saturated mercuric chloride for methane and ethylene measurements by gas chromatography, while the remaining replicate was killed for MIMS oxygen measurements.

Wild type *P. stutzeri* HI00D01 cultures evolved methane from the degradation of methyl phosphonate and methane and ethylene from the degradation of semi-labile DOM polysaccharides (Table 1). In contrast, the mutant strain *phnK*491::Tn*5* cultures did not produce methane or ethylene in the presence of methyl phosphonate or semi-labile DOM polysaccharides, indicating that the C–P lyase pathway was disrupted by the transposon insertion (Table 1). Hydrocarbon gas evolution proceeded under aerobic conditions, as indicated by the dissolved oxygen concentrations remaining in samples at the end of the experiment, which remained at approximately 70% of the initial dissolved oxygen concentration (Supplementary Table 2). In addition, both wild type and mutant *phnK*491::Tn*5* cultures were viable during the incubation period, as indicated by the similar increases in cell growth during the experiment, approximately 3.5 fold and 1.8 fold, respectively (Supplementary Table 2). These results indicate that the phosphonates associated with semi-labile DOM polysaccharides are bio-available and can partially support the phosphorus demand of bacteria harbouring C–P lyase.

Data availability. The authors declare that all data supporting the findings of this study are available within the article and its Supplementary Information files. *Pseudomonas stutzeri* HI00D01 Whole Genome Shotgun data has been deposited at DDBJ/ENA/GenBank under the accession LWEF00000000. The version described in this paper is version LWEF01000000.

References

31. Karl, D. K. & Tien, G. MAGIC: a sensitive and precise method for measuring phosphorus in aquatic environments. *Limnol. Oceanogr.* **37,** 105–116 (1992).
32. Repeta, D. J. in *Biogeochemistry of Dissolved Organic Matter* (eds Hansell, D. A. & Carlson, C. A.) 21–63 (Academic, 2015).
33. Ferrón, S., Wilson, S. T., Martínez-García, S., Quay, P. D. & Karl, D. M. Metabolic balance in the mixed layer of the oligotrophic North Pacific Ocean from diel changes in O_2/Ar saturation ratios. *Geophys. Res. Lett.* **42,** 3421–3430 (2015).
34. Kana, T. M. *et al*. Membrane inlet mass spectrometer for rapid high-precision determination of N_2, O_2, and Ar in environmental water samples. *Anal. Chem.* **66,** 4166–4170 (1994).
35. Kana, T. M., Cornwell, J. C. & Zhong, L. Determination of denitrification in the Chesapeake Bay from measurements of N_2 accumulation in bottom water. *Estuar. Coast* **29,** 222–231 (2006).
36. García, H. E. & Gordon, L. I. Oxygen solubility in seawater: better fitting equations. *Limnol. Oceanogr.* **37,** 1307–1312 (1992).
37. Hamme, R. C. & Emerson, S. R. The solubility of neon, nitrogen and argon in distilled water and seawater. *Deep-Sea Res. I* **51,** 1517–1528 (2004).
38. Sosa, O. A., Gifford, S. M., Repeta, D. J. & DeLong, E. F. High molecular weight dissolved organic matter enrichment selects for methylotrophs in dilution to extinction cultures. *ISME J.* **9,** 1–15 (2015).
39. Lalucat, J., Bennasar, A., Bosch, R., García-Valdés, E. & Palleroni, N. J. Biology of *Pseudomonas stutzeri*. *Microbiol. Mol. Biol. Rev.* **70,** 510–547 (2006).
40. White, A. K. & Metcalf, W. W. Two C–P lyase operons in *Pseudomonas stutzeri* and their roles in the oxidation of phosphonates, phosphite, and hypophosphite. *J. Bacteriol.* **186,** 4730–4739 (2004).
41. Errey, J. C. & Blanchard, J. S. Functional annotation and kinetic characterization of PhnO from *Salmonella enterica*. *Biochemistry* **45,** 3033–3039 (2006).
42. Hover-Jensen, B., McSorley, F. R. & Zechel, D. L. Catabolism and detoxification of 1-aminoalkylphosphonic acids: N-acetylation by the phnO gene product. *PLoS ONE* **7,** e46416 (2012).
43. Metcalf, W. W. & Wanner, B. L. Mutation analysis of an *Escherichia coli* fourteen-gene operon for phosphonate degradation, using TnphoA′ elements. *J. Bacteriol.* **175,** 3430–3442 (1993).