Single-cell bacterial transcription measurements reveal the importance of dimethylsulfiniopropionate (DMSP) hotspots in ocean sulfur cycling.
Single-cell bacterial transcription measurements reveal the importance of dimethylsulfoniopropionate (DMSP) hotspots in ocean sulfur cycling

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Dimethylsulfoniopropionate (DMSP) is a pivotal compound in marine biogeochemical cycles and a key chemical currency in microbial interactions. Marine bacteria transform DMSP via two competing pathways with considerably different biogeochemical implications: demethylation channels sulfur into the microbial food web, whereas cleavage releases sulfur into the atmosphere. Here, we present single-cell measurements of the expression of these two pathways using engineered fluorescent reporter strains of *Ruegeria pomeroyi* DSS-3, and find that external DMSP concentration dictates the relative expression of the two pathways. DMSP induces an upregulation of both pathways, but only at high concentrations (>1 μM for demethylation; >35 nM for cleavage), characteristic of microscale hotspots such as the vicinity of phytoplankton cells. Co-incubations between DMSP-producing microalgae and bacteria revealed an increase in cleavage pathway expression close to the microalgae’s surface. These results indicate that bacterial utilization of microscale DMSP hotspots is an important determinant of the fate of sulfur in the ocean.

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Upt to 10% of the carbon fixed by phytoplankton cells in the ocean is converted to dimethylsulfiniopropionate (DMSP)\(^1\), resulting in a global production of this compound that exceeds one billion tons per year\(^2\). DMSP is an important currency in the ecological and metabolic exchanges between phytoplankton and heterotrophic bacteria\(^3\), as it represents a major nutrient source that contributes significantly to the sulfur and carbon demand of bacteria (up to 95% and 15%, respectively)\(^4,5\). DMSP is utilized by marine bacteria via two competing catabolic pathways\(^6\): the demethylation pathway leads to the incorporation of both carbon and sulfur into bacterial biomass, whereas the cleavage pathway results in the utilization of carbon but the release of sulfur in the form of the climatically sensitive gas dimethylsulfide (DMS). The environmental factors that govern the utilization of one pathway over the other, and ultimately the production and release of DMS to the atmosphere, have remained elusive, marking a major gap in the mechanistic link between microbial processes and global-scale carbon and sulfur biogeochemical cycles.

The water-column concentration of DMSP has been hypothesized to be an important factor regulating the choice of degradation pathway by bacteria (DMSP Availability Hypothesis\(^6\)) and it has been speculated that bacteria control the fate of sulfur from DMSP by adjusting the relative expression of the demethylation and cleavage pathways (Bacterial Switch Hypothesis\(^7\)). Concentrations of DMSP in bulk seawater are typically low, ranging from a few nanomolar (global oceanic average: 16.91 ± 22.17 nM\(^8\)) up to 200 nM during phytoplankton blooms\(^9\). However, much higher DMSP concentrations are expected to occur in the vicinity of individual DMSP-producing organisms, such as phytoplankton cells, which can have intracellular DMSP concentrations of hundreds of millimolar\(^10\). Efforts to elucidate the environmental drivers of microbial catabolism of DMSP have to date been limited to measurements in large-volume batch cultures\(^11,12\) and seawater samples\(^13\). As a consequence, an understanding of the influence of microscale heterogeneity in DMSP concentrations on the microbial choice of degradation pathway is lacking.

Here, we report that the external concentrations of DMSP that are relevant for controlling the expression of degradation pathways by a model copiotrophic bacterium are unexpectedly high, and are characteristic of DMSP hotspots. This finding was enabled by the development of the first single-cell, time-resolved measurements of DMSP degradation pathway expression and their application to study the response of bacteria to different concentrations of DMSP.

**Results and discussion**

**Construction and validation of fluorescent reporter bacteria.** To examine the relative expression of bacterial DMSP catabolism genes at the single-cell level, we genetically transformed *Ruegeria pomeroyi* DSS-3, a model Alphaproteobacterium from the Roseobacter clade. Like many members of the Roseobacter clade, which plays a central role in DMSP cycling\(^14\), *R. pomeroyi* harbors both DMSP catabolic pathways\(^15\). We transformed *R. pomeroyi* cells with a custom-built tricolor promoter-fusion plasmid designed to simultaneously report metabolic activity and the expression of the genes encoding the two DMSP degradation pathway enzymes through different fluorescence emission (Fig. 1a, b). In the engineered plasmid, the promoter regions of DMSP-dependent demethylase (DmdA) and DMSP lyase (DddW), which catalyze the first steps of the demethylation and cleavage pathways, respectively, control the expression of fluorescent proteins (Methods). Out of the three functional DMSP lyases (DddP, DddQ, and DddW)\(^16\) encoded in the *R. pomeroyi* DSS-3 genome, DddW was chosen in this study due to its strong upregulation response to DMSP reported in previous transcriptomic studies\(^11,17\), which suggests that it is the primary DMSP lyase in this bacterium. However, some of the cleavage dynamics, controlled by DddP and DddQ, may be missed by our approach.

Construction of promoter-fusion reporter strains, followed by quantitative single-cell time-lapse microscopy, has been commonly adopted to utilize fluorescence signal dynamics as a proxy for native gene expression behaviors\(^18-20\). To control for signal bias caused by the choice of fluorescent protein fused to each promoter region, we constructed two *R. pomeroyi* reporter strains (Goofy and Regular), for which we interchanged the color of fluorescent proteins fused to the *dmdA* and *dddW* promoter regions (Fig. 1a, b and Supplementary Fig. 1). The choice of fluorescent protein led to some differences in the temporal evolution of fluorescence signal, but did not affect our overall conclusions (Supplementary Fig. 2). A comparison of fluorescence signal output by tricolor and single-color reporter strains confirmed that promoter fusion cassettes induce fluorescent protein expression whether encoded alone or together (Supplementary Fig. 2).

To confirm that the strains specifically report *dmdA* and *dddW* gene expression, and to test for non-specific responses, the engineered bacteria were incubated with seven different carbon sources. Rich medium (5% 1/2 YTSS), propionate, acetate, succinate, and glucose did not elicit any fluorescence response (Supplementary Fig. 3). Glucose was chosen as the most suitable negative control for subsequent experiments for the following reasons: it elicited no non-specific DMSP gene transcription responses (Supplementary Fig. 3); its molecular weight is similar to DMSP; and the metabolic pathways of glucose and organosulfur compounds are distinct. The only carbon sources that led to an increase in cell fluorescence were DMSP (activating both *dmdA* and *dddW* promoters) and acrylate, a known *dmdA* inducer\(^21\) (activating only *dmdA*, but not *dddW*), thus confirming the validity of our reporter construct design (Supplementary Fig. 3).

**Time-lapse DMSP incubation experiments in microfluidic chips.** A custom microfluidic chip containing nine observation chambers was employed for the simultaneous incubation of an engineered reporter strain with a range of concentrations of DMSP as a sole amended carbon source (Fig. 1c). The absence of fluid flow in the observation chambers enabled us to monitor the expression of DMSP degradation pathways in a time-resolved manner at the single-cell level (Fig. 1d). Images in phase contrast and fluorescence (in red, yellow, and teal channels) were acquired every 45 min for 24 h at six or seven positions per observation chamber (Fig. 2), encompassing 218 × 120 (mean ± s.d.) cells per field of view (at t = 45 min) per condition (Supplementary Fig. 4). Microscope and camera settings were optimized to minimize phototoxicity and photobleaching while maximizing fluorescence signal capture.

Low levels of expression of both pathways occurred even in the absence of DMSP (Supplementary Fig. 5 and Supplementary Note 1), with baseline *dmdA* expression 1.0–6.7 times higher than *dddW* expression (Fig. 3). High variability of fluorescence output among replicate experiments at ≥10 μM DMSP (Supplementary Fig. 6), likely caused by slight differences in subculture growth phase, prevented the comparison of pathway reporters within each color (Supplementary Fig. 5). Thus, across-color ratio calculation, which enabled comparisons of pathway expression within the same experiment, was employed in our study (Fig. 3). Importantly, the across-color ratios (0.15–1.0; Fig. 3) and
bacteria start to increase approximates the threshold DMSP concentration above which translation as the major fate of DMSP in seawater22, our results also levels. Consistent with existing evidence that points to demethylation14, this increase plateaued above 100 μM, 75 μM, and 1 mM conditions, but decreased as the DMSP magnitude of the end-point gene expression returns to baseline levels. We therefore used the stability of fluorescent proteins (half-lives of hours to more than a day23), the fluorescence signal is expected to persist even after gene expression returns to baseline levels. Therefore, we used the maximum gene expression levels of dddW and dmdA. Normalized maximum gene expression levels of both pathways increased approximately linearly with DMSP concentration between 1 and 75 μM DMSP (0.07–0.77 a.u. for dmdA; 0.02–0.62 a.u. for dddW; Fig. 2c, d). This increase plateaued above 100 μM (Fig. 2c, d), possibly as a result of the gene expression machinery becoming saturated and unable to respond as sensitively to DMSP at these high concentrations (Supplementary Note 2).

To determine how consumption of DMSP in the chambers may have affected our conclusions, we performed a larger-volume (8 ml) experiment in which we directly measured DMSP concentration and cell fluorescence, for selected timepoints (0, 2, 8, 24 h) and initial DMSP concentrations (1 μM, 75 μM, 1 mM). DMSP concentration decreased over time, due to uptake by bacteria (Supplementary Fig. 10). Consistent with results from the microfluidic chip experiments (Fig. 2), the initial rate of increase in the fluorescent signal was conserved between the 75 μM and 1 mM conditions, but decreased as the DMSP concentration diminished due to bacterial uptake (Supplementary Fig. 10). The saturation of the fluorescent signal coincided temporally with the depletion of DMSP (at 8 h, for the 75 μM condition; Supplementary Fig. 10). These results suggest that cells initially increase gene expression at a rate that is independent of DMSP concentration, but halt their gene upregulation when the DMSP supply is exhausted.

Rather than expressing only one pathway at any given DMSP concentration, as implied by the Bacterial Switch Hypothesis7, we observed that bacteria express both pathways simultaneously, but modulate the ratio of cleavage and demethylation according to DMSP concentration (Fig. 3). Overall, the cleavage-to-
demethylation expression ratio increased with DMSP concentration up to 100 μM, above which it started to plateau (Fig. 3b). Baseline expression levels that are biased towards demethylation were represented by cleavage-to-demethylation ratios of 0.15–1.0 in the glucose negative controls (Fig. 3). At high DMSP concentrations between 10 μM and 1 mM, bacteria gradually skewed their gene expression towards the cleavage pathway, with the cleavage-to-demethylation ratio increasing from 1.89 ± 0.29 at 10 μM to 8.10 ± 0.60 at 1 mM (Fig. 3, strain Regular). Similar ratio values were obtained with strain Goofy (Fig. 3). These results indicate that the cleavage pathway becomes more strongly expressed than the demethylation pathway above a transition concentration of DMSP that lies between 1 and 10 μM. We propose that at this transitional concentration, the sulfur needs of the bacteria are completely met through the demethylation pathway, and excess organic sulfur at higher DMSP concentrations is released as DMS via cleavage.

**Raman microspectroscopy.** The effect of sulfur satiation on cleavage pathway expression was more directly observed via single-cell Raman microspectroscopy. Measurements with deuterium-labeled DMSP revealed that bacteria that were satiated in sulfur through prior exposure to methionine (also an organic sulfur source) maintained uptake of DMSP but skewed gene expression toward the cleavage pathway (Supplementary Fig. 11). Bacterial sulfur demand has been proposed as a factor that regulates the fate of DMSP6,7,12. Since different elements of the DMSP molecule are harvested by the bacteria through demethylation (both carbon and sulfur) and cleavage (carbon...
and microbial pathway choice in the ocean. To determine DMSP degradation gene expression in the context of microscale hotspots, we exposed the *R. pomeroyi* fluorescent reporter strains to an ecologically relevant point source of DMSP: a unicellular phytoplankton. Concentration gradients of nutrients, often including DMSP, are present in the microenvironment directly surrounding phytoplankton cells (the phycosphere24). We co-incubated the reporter strains with the unicellular dinoflagellate *Breviolum CCMP2459*, which belongs to a family containing some of the most prolific producers of DMSP (Symbiodinaceae; with intracellular DMSP concentrations of 36–7590 mM)10.

Co-incubations were performed on agarose pads, which immobilized both phytoplankton and bacterial cells for ease of observation. After 24 h of co-incubation in the dark, high-magnification (100× objective) epifluorescence microscopy images of the phycosphere surrounding individual *Breviolum* cells were acquired (Fig. 4a, b and Supplementary Fig. 12). To avoid alterations to the phycosphere due to microscopy light-induced cellular stress, images were acquired at a single time point (24 h). Only real fluorescence was quantified to represent bacterial pathway expression (due to spectral leakage in the red fluorescence channel by photosynthetic pigments, e.g., chlorophyll and carotenoids), with strain Regular reporting demethylation (*dmdA*; *n* = 15 *Breviolum* cells) and strain Goofy reporting cleavage (*dddW*; *n* = 18 *Breviolum* cells) (Fig. 4). Fluorescence intensities of bacteria were averaged across *Breviolum* cells as a function of distance from the phytoplankton cell (Supplementary Note 3).

*R. pomeroyi* gene expression patterns reflected the spatial locations of the bacteria within the phycosphere of *Breviolum* cells. According to modeled DMSP diffusion assuming a leakage rate of 11% of intracellular DMSP per day (Supplementary Note 4), the steady-state concentration at the surface of a *Breviolum* cell (*radius = 3.3 ± 0.9 μm, mean ± s.d.) was 197 nM and decayed exponentially with distance, *r*, from the center of the phytoplankton cell (Supplementary Fig. 13). In line with the predicted DMSP concentration profile within the phycosphere, bacteria that were nearest to the surface of *Breviolum* cells, but far enough not to be affected by spectral interference from photosynthetic pigments (*r* = 7.4 μm), were the most metabolically active, exhibiting YFP fluorescence intensities that were on average double (1.4 ± 0.5 a.u., Goofy; 0.4 ± 0.2 a.u., Regular) those exhibited by bacteria located at *r* = 18.6 μm (0.7 ± 0.3 a.u., Goofy; 0.2 ± 0.05 a.u., Regular), beyond which YFP intensities did not change with distance (Fig. 4c, d).

The expression of the cleavage pathway (*dddW*) also increased with decreasing distance from a *Breviolum* cell. The *dddW* expression levels (3.4 ± 1.1 × 10⁻² a.u., raw TFP signal) were highest near the surface of *Breviolum* (*r* = 7.4 μm; modeled DMSP concentration = 89 nM) (Fig. 4d) and decreased to baseline levels (2.4 ± 0.4 × 10⁻² a.u., raw TFP signal) at *r* ≥ 18.6 μm (modeled DMSP concentration = 35 nM). These results differ from our previous microfluidic experiments where exposure to pure DMSP at <1 μM did not lead to *dddW* fluorescence upregulation (Supplementary Fig. 7). This discrepancy may be due to the presence of compounds in algal exudates that positively influence the regulation of *dddW* expression, or by the greater sensitivity of the camera setup used in the *Breviolum* experiment. In contrast, the demethylation pathway (*dmdA*) was not expressed above baseline levels at any distance from the *Breviolum* cells (Fig. 4c), but its expression, normalized by the average baseline YFP intensity (proxy for metabolic activity), was still higher than that of the cleavage pathway throughout the phycosphere (Fig. 4e). As a result, relative pathway expression was skewed towards demethylation at all distances from a *Breviolum* cell (Fig. 4f), but with decreasing distance, and thus increasing DMSP concentration, the cleavage-to-demethylation
pathway ratio increased in a pattern consistent with the microfluidic observations (Fig. 3a).

These results suggest that within the phycosphere of a small phytoplankton cell slowly exuding DMSP, elevated production of DMS due to cleavage by marine bacteria occurs close to the surface of the phytoplankton cell, but most of the DMSP within the phycosphere is degraded through the demethylation pathway. However, in the scenario of a lysing phytoplankton cell that releases its intracellular DMSP at once, for example at the demise of a phytoplankton bloom, DMSP within the phycosphere can reach micro- or millimolar concentration for seconds to minutes26. Matching these time scales, bacterial gene expression (transcription and translation) can theoretically be upregulated within a few minutes27, although limitations in fluorescence signal detection prevented the observation of such early responses in the present study. Furthermore, oligotrophic DMSP degraders
such as SAR11\(^ {28,29}\) likely employ regulatory mechanisms that differ from copiotrophic bacteria such as \( \textit{R. pomeroyi} \). In general, the results from our microfluidic experiments suggest that in the vicinity of lysing phytoplankton cells (>10 \( \mu \text{M} \)) or in other microenvironments with similarly high DMSP levels\(^ {30}\), both DMSP degradation genes increase expression, with cleavage more so than demethylation. These microscale dynamics are consistent with macroscale patterns of elevated DMS production that are observed during the decline of phytoplankton blooms\(^ {31}\) and following high rates of viral-induced phytoplankton lysis\(^ {22}\).

Taken together, our observations reveal that the metabolic machinery of DMSP-degrading copiotrophic bacteria may be adapted for encounters with DMSP hotspots. Baseline expression of both pathways was detected even in the absence of DMSP (Supplementary Fig. 5), possibly owing to promoter leakage of \( \text{dmdA} \) and \( \text{dddW} \), which likely allows bacteria to be poised for the next encounter with DMSP. Upregulation of DMSP degradation genes, beyond baseline levels, was only observed at high DMSP concentrations that are characteristic of hotspots: above 1 \( \mu \text{M} \) for \( \text{dmdA} \) (Fig. 2a and Supplementary Fig. 6) and above 35 \( \mu \text{M} \) for \( \text{dddW} \) (Fig. 4b, d and Supplementary Fig. 13). Furthermore, \( K_m \) values of DMSP degradation enzymes (5.4 \( \mu \text{M} \) for \( \text{DmdA} \)\(^ {33}\) and 4.50–8.68 \( \mu \text{M} \) for \( \text{DddW} \)\(^ {34}\)) are orders of magnitude above the mean seawater concentrations of DMSP (16.91 ± 22.17 \( \text{nM} \))\(^ {8}\), further supporting the notion that bacteria are adapted to exploit sporadic encounters with DMSP hotspots\(^ {35}\) as we observed by single-cell imaging.

Identifying the environmental determinants of microbial DMSP cycling is key in understanding their effects on global climate and biogeochemical cycles. Two interconnected concepts, the DMSP Availability Hypothesis\(^ {9}\) and the Bacterial Switch\(^ {7}\), were proposed nearly two decades ago to explain the interplay between the two DMSP degradation pathways and the factors leading to the production of DMS, but have remained largely hypothetical. The present study offers the first direct evidence that the ambient concentration of DMSP regulates the relative (i.e., cleavage-to-demethylation ratios; Figs. 3, 4), rather than mutually exclusive, expression of demethylation and cleavage pathways. We observed that elevated concentrations of DMSP (>10 \( \mu \text{M} \)), which are typically found in microscale hotspots, shift bacterial DMSP degradation toward cleavage and are ultimately expected to increase the bacterial production and release of DMS. Thus, we propose that the concentrations of DMSP that are most relevant for the bacterial production of DMS, and ultimately for global sulfur cycling and for the production of DMSP-derived cloud condensing nuclei, may not be the levels present in bulk seawater, but instead those existing in microscale hotspots.

This points to the importance of understanding the relative contribution of DMSP catalabilism rates in hotspots compared to the bulk seawater, and the need to develop more realistic microscale methods to quantify the utilization and fate of this ubiquitous and important marine compound.

**Methods**

**Construction of tricolor fluorescent reporter strains.** Tricolor fluorescent reporters were constructed in the marine model organism \( \textit{R. pomeroyi} \) DSS-3 (wild-type strain, a gift from Prof. M. A. Moran, University of Georgia) to visually report expression of DMSP degradation genes (\( \text{dddW} \) and \( \text{dmdA} \)). \( \text{DddW} \) was chosen due to its strong upregulation response to DMSP reported in previous transcriptomic studies\(^ {11,17}\), which suggests that \( \text{DddW} \) is the primary DMSP lyase in \( \textit{R. pomeroyi} \) DSS-3. Three fluorescent proteins were chosen for brightness, monomeric structures, and spectral separation: mTFP1 (teal)\(^ {36}\); mVenus-Q69M (yellow)\(^ {37}\), which is the mVenus YFP\(^ {38}\) modified with a Q69M mutation to reduce environmental sensitivity; and mKate2 (far-red)\(^ {39}\). To control for bias caused by the choice of color of fluorescent protein (RFP or TFP) fused to each promoter region, we constructed two \( \textit{R. pomeroyi} \) reporter strains (Goofy and Regular) in which we interchanged the fluorescent proteins fused to \( \text{dddW} \) and \( \text{dmdA} \) promoter regions (Fig. 1a, b).

Three promoter fusion cassettes were inserted into a single vector backbone (pBRR1MCS-2, a 5.144 kb, broad-host-range, medium copy number plasmid with a kanamycin resistance cassette and origin of replication pBRRI, originally isolated from \( \textit{Bordetella bronchi (e)ptic a} \))\(^ {40}\) to enable gene expression readouts from individual cells (Supplementary Fig. 14): a \( \text{dmdA} \) promoter reporter cassette; an \( \text{dddW} \) promoter reporter cassette; and a constitutively expressed \( \text{yfp} \) cassette (Fig. 1a, b). The 500 bp sequence upstream of the \( \text{dddW} \) gene and 222 bp upstream of the \( \text{dmdA} \) gene in the \( \textit{R. pomeroyi} \) DSS-3 genome were determined as putative promoter regions and used to construct promoter reporter cassettes. A strong, constitutive synthetic promoter P\(_{E.coli lac} \)(an \( E. coli lac \) promoter derivative)\(^ {42,43}\) controlled the expression of YFP, whose intensity was utilized as a proxy for cell viability, plasmid copy number, and metabolic activity (Supplementary Fig. 15). Transcriptional terminators (RNAl, TSAl, TR2-17, TL17, BS7, and TS7+E-) and spacer regions between promoter fusion cassettes were cloned from plasmid pZS2-123\(^ {44}\). As an intermediate step in the construction of the tricolor reporter strains, a derivative of pZS2-123 (with its original promoters replaced with 500 bp sequence upstream of \( \text{dmdA} \) or \( \text{dddW} \) genes), pZS2-200, was built using restriction enzymes: AvrII, Xmal, Xhol, BamHI, XmnI, and Sall (New England Biolabs).

**Construction of control reporter strains.** Truncated versions of the tricolor reporters (Supplementary Fig. 1), each of which contained one of the promoter fusion cassettes, were built to test the effect of including three promoter fusion cassettes within one DNA construct (Supplementary Fig. 2). We also constructed constitutively fluorescent, single-color \( \textit{R. pomeroyi} \) strains (Supplementary Fig. 1) to quantify spectral leakage amongst fluorescent protein colors, and to calculate the spectral leakage correction matrix, \( B \) (Supplementary Note 1 and Supplementary Fig. 16).
DNA Transformation of R. pomeroyi through conjugation. Reporter plasmids were transformed into R. pomeroyi DSS-3 through a triparental conjugation method, which was found to be ideal due to the large sizes of our reporter plasmids (up to 7,974 bp). The plasmid size of wild-type R. pomeroyi was prepared in half-strength YTSS (1/2 YTSS) containing (500 ml−1)2 yeast extract (BD Biosciences), 1.25 g tryptone (BD Biosciences), 10 g sea salts (Sigma-Aldrich). In addition, overnight liquid cultures of helper E. coli containing the pRK600 plasmid (15 μg ml−1) and donor E. coli containing a constructed reporter plasmid (50 μg ml−1) were prepared in LB broth cultures. E. coli cultures used as PCR templates were prepared in half-strength YTSS (1/2 YTSS) medium to eliminate the multiple cloning site (MCS) within the lacZ gene in the pBR104MCS-2 vector to prevent fusion with the β-galactosidase α-peptide. Two extra stop codons (TAA- TAA) were added to each fluorescent protein gene sequence through primer design.

DNA fragments were assembled using NEBuilder HiFi DNA Assembly Master Mix (New England Biolabs). Assembled plasmids were transformed into electrocompetent E. coli (NEB 10-beta; New England Biolabs) through electroporation (Gene Pulser Xcell, Bio-Rad), positive colonies were picked on X-gal/PTG Luria Broth (LB) plates, and correct assembly of DNA fragments was confirmed through sequencing of purified plasmids using diagnostic primers listed in Supplementary Table 2.

Bacterial culture preparation for experiments. A frozen glycerol stock of each R. pomeroyi reporter strain was streaked onto a half-strength YTSS (1/2 YTSS) culture plate containing (500 ml−1)2 yeast extract (5.7 g) (Bacto Bacto Agar, BD Biosciences), amended with 200 μl of washed overnight culture of helper E. coli, and 200 μl of washed overnight culture of donor E. coli. 50 μl of this bacterial mixture was spotted onto a 1/2 YTSS plate, and incubated overnight at 30 °C to allow mating to occur. Selection for plasmid-containing R. pomeroyi was achieved by re-streaking onto a 1/2 YTSS plate amended with kanamycin (50 μg ml−1) and potassium tellurite (50 μg ml−1; Fluorochem). Like many marine microorganisms, R. pomeroyi was found in this study to be resistant to potassium tellurite, while E. coli is known to be sensitive to the oxide mineral. Successfully transformed R. pomeroyi were confirmed through colony PCR and sequencing.

Carbon sources test. To assess the validity of engineered reporter strains, as well as to identify an appropriate negative control carbon source, R. pomeroyi reporter strains Regular and Goofy were incubated with a range of carbon sources, and their fluorescence response and growth were measured (Supplementary Fig. 3). Carbon sources chosen for this experiment were utilized in previous studies to cultivate R. pomeroyi. MBM solutions amended with 10 mM of the following carbon sources were prepared: DSS (Tokyo Chemical Industry), sodium succinate dibasic hexahydrate (Sigma-Aldrich), sodium propionate (Sigma-Aldrich), sodium acetate (Sigma-Aldrich), sodium acrylate (Sigma-Aldrich), or δ-glucose (Sigma-Aldrich). MBM solutions were filter sterilized (0.2 μm) after dissolution of carbon sources.

The 9% 1/2 YTSS was prepared with a dilution (1/20 vol/vol) of the rich medium in non-carbon amended MBM. The 9% 1/2 YTSS reporter strains on agar plates were washed and resuspended in non-carbon amended MBM. Resuspended cells (2 μl) were seeded into 0.75 ml of each carbon source MBM solution amended with 25 μl−1 kanamycin. Incubations were performed in 2 ml microcentrifuge tubes (Eppendorf) in the dark at 30 °C with 200 rpm orbital shaking for 18.5 h before fluorescence imaging.

Glucose was chosen as the most suitable negative control for the following reasons: it elicited no non-specific DMSP gene transcription response (Supplementary Fig. 3); its molecular weight is similar to DMSP; and the metabolic products of glucose and its heme-soluble compounds are distinct. The low DMSP concentration (≤1 μM) experiment (Supplementary Fig. 7) was the only instance in which sucinate was used as the negative control, as it had been utilized in previous studies21,49. While sucinate was also suitable as negative control (Supplementary Fig. 3), it produced slightly higher non-specific fluorescence response than glucose; thus, glucose, unless otherwise noted, was utilized as the negative control for all other experiments.

Microfluidic device fabrication. The microfluidic device containing nine parallel observation chambers (Fig. 1c) was fabricated using soft lithography.9 A mold for the observational chamber geometry was fabricated with SU8 on a silicon wafer. The microfluidic device was then created by casting polydimethylsiloxane (PDMS) (SYLGARD 184 Silicone Elastomer Kit; Dow Corning) onto the mold. The cured PDMS was then removed from the mold, perforated with inlet and outlet holes with a biopsy punch (1.5 mm diameter), and permanently fixed to a glass cover slide (60 mm × 24 mm; 0.17 ± 0.005 mm precision thickness; Carl Roth) by plasma bonding. Depth of each observation chamber was ~60 μm.

Microscopy. All experiments were performed using an inverted epifluorescence TE2000 microscope (Nikon) controlled through Nikon Elements software (unless otherwise specified). A Spectra X LED light source (Lumencore) provided single wavelength excitation illumination for fluorescence imaging (100% LED power multiplied). For the Particles in Focus System imaging experiment, Nikon Elements software was used to maintain focus in time-lapse experiments. Three filter cubes (Chroma) were used for fluorescence imaging: a custom filter cube optimized for mKate2 RFP (ET580/ 25x excitation filter, T600lp band-pass filter, and ET645/75m emission filter), Chroma 49093 for YFP, and Chroma 49013 for TFP. Unless otherwise indicated, the time-lapse filters for each condition were chosen to maximize signal capture. At each field of view, phase contrast and fluorescence images were captured sequentially in the following order: phase contrast, red fluorescence channel (575 nm excitation), yellow fluorescence channel (508 nm excitation), and teal fluorescence channel (440 nm excitation). Bacteria were introduced into microfluidic devices, allowed to settle for 20–30 min, and imaged at the plane of the glass coverslip surface.

Images were acquired with an electron multiplying CCD (EMCCD) camera (iXon; Andor Technology) (600 x 600 pixels; 8 μm pixel size) for the following experiments: time-lapse DMSP experiments in microfluidic chips large volume DMSP concentration measurement experiment (Supplementary Fig. 10); carbon sources test (Supplementary Fig. 3); DMSP uptake experiment (Supplementary Fig. 11); and phytoplankton co-incubation experiment (Fig. 4 and Supplementary Fig. 12). For time-lapse DMSP experiments in microfluidic chips and the large-volume DMSP concentration measurement experiment, a 40× objective (CFI Plan Fluor ELWD Achromatic NA 0.40, corrected for oil immersion to 0.17; Nikon) was used with electron multiplier gain at 3×, and the following exposure times: phase contrast (20 ms, 5% white LED power), red (100 ms), yellow (100 ms), and teal (200 ms). Imaging conditions for the carbon sources test were identical, except exposure time for teal fluorescence imaging was 100 ms. Time-lapse imaging for the DMSP uptake experiment was done in parallel with, but on a different microscope from, Raman microspectroscopy measurements (see below). Only phase contrast and teal fluorescence were acquired, with microscopy setup as described above. For phytoplankton co-incubation imaging, an oil-immersion 100× objective (CFI Plan Apo Lambda DM 100× Oil; Nikon) was used, without electron multiplication, with the following exposure times (total 240 ms per image) and LED powers: phase contrast, 60 ms, 10%; red, 100 ms, 100%; yellow, 40 ms, 50%; and teal, 40 ms, 100%.
Finally, images for the low DMSP concentration (≤1 μM) experiment (Supplementary Fig. 7) were acquired with an sCMOS camera (Zyla 4.2, Andor Technology) (2048 × 2048 pixels; 6.5 μm pixel size). A 40× objective (described above) was used, with the following camera exposure times: phase contrast, 9.8 ms, 10% white LED power; and all fluorescence channels, 200 ms.

Image analysis. Analysis of fluorescence images was performed in MATLAB (MathWorks) using an automated image segmentation and fluorescence quantification software developed in-house. Detailed descriptions of image processing and analysis methodologies for microfluidic and agarose pad co-culture experimentation are provided in Supplementary Notes 1 and 3, respectively. Briefly, cells were segmented by pixel intensity thresholding in phase contrast images. Background subtraction and spectral leakage correction were performed to enable accurate quantification of cellular fluorescence. Thresholding on YFP fluorescence intensity (reflecting cellular activity) was applied to only include intact cells for further analyses. Finally, fluorescence signals in red and teal channels of each cell were normalized by the mean YFP signal at each time point of each experimental condition.

DMSP pathway expression time-lapse experiment. Each replicate experiment represents a biological replicate performed on a single microfluidic device containing nine observation chambers (Fig. 1c). For each replicate experiment, one of the two R. pomeroyi reporter strains (Regular or Goofy) was prepared for experimentation as described above. At the end of subculture incubation, cells were washed and concentrated by 4.5× in non-carbon amended MBM amended with kanamycin (10 μg ml⁻¹) and distributed into nine separate microcentrifuge tubes (Eppendorf). Each treatment was subjected to a final concentration of glucose or DMSP, a 10× concentrated stock solution was prepared in non-carbon amended MBM amended with kanamycin (10 μg ml⁻¹). To initiate incubation, 10× stock solutions were diluted to 1× final concentration in the cell-containing MBM, resulting in a 4.05× cumulative concentration of subcultured cells. Each observation chamber was populated with 12.5 μl of treated cells. Inlet and outlet holes of observation chambers were sealed with clear tape to minimize evaporation. Since PDMS is a gas-permeable material, oxygen is not expected to be limited in our experimental setup. Cells in observation chambers were allowed to settle onto the glass coverslips surface with gravity for 20–30 min before initiation of image capture. Phase contrast and fluorescence images were captured at seven positions, determined manually before start of imaging, per observation chamber every 45 min for ~24 h. Repeat experiment 3 of strain Regular (Supplementary Figs. 4, 6, 15) contained 6 imaging positions (instead of 7) per observation chamber. All fluorescence kinetic experiments, except the low DMSP concentration (<1 μM) experiment only (Supplementary Fig. 7), 1 mM succinate was used as negative control, all experimental conditions contained 50 μg ml⁻¹ kanamycin, and fluorescence was monitored by microscopy with image acquisition every 30 min for 7.4 h with a cage incubator set to 30 °C.

Cultivation of phytoplankton. The dinoflagellate Brevisolum (strain CCMIP2459, formally within genus Symbiodinium⁴¹) was chosen for its prolific production of DMSP. Brevisolum cells were grown in sterile culture flasks (Nunc EasyFlasks, 25 cm³ volume; Thermo Fisher Scientific) under a diel light cycle (14 h light:10 h dark, (100 μmol m⁻² s⁻¹)) in 30 ml f/2 medium at 22 °C. Cells at 22 days post-inoculation (a 1:100 dilution into fresh medium) were harvested for experimentation at 14:00 in the afternoon. Cellular concentration was determined by counting in a microfluidic observation chamber (21 μl⁻¹).

Phytoplankton-bacteria co-incubation experiment. Co-incubations between Brevisolum cells and R. pomeroyi reporter strains Regular or Goofy were performed on agarose pads, which immobilized the algal cells and allowed them to establish their phycospheres (i.e., the immediate regions surrounding unicellular algae cells) through steady exudation. For agarose pad preparation, low melting temperature agarose (2.5%) was dissolved in sterile water at 1/3× the final concentration and gently dissolved in a microwave. After partial cooling, kanamycin was added at 25 μg ml⁻¹ final concentration. Rubber gaskets (0.5 mm thickness) were manually cut into square frames (~2 cm × 2 cm inner square area) and placed on glass coverslips (22 mm × 50 mm; VWR). The inner square areas of rubber gaskets were filled with ~500 μl melted agarose–kanamycin mixture. Agarose pads were allowed to cool and solidify for 1.5 h before seeding with R. pomeroyi strains.

Bacteria were first seeded onto agarose pads and allowed to grow for 24 h without phytoplankton. Overnight cultures of R. pomeroyi strains grown in 1/2 YTSS were washed and concentrated threefold in non-carbon amended MBM amended with kanamycin (10 μg ml⁻¹). Randomly selected bacterial culture plate material was mixed and spotted onto the center of each agarose pad, and loosely covered with a plastic lid (without contacting the agarose) to minimize evaporation. R. pomeroyi strains were allowed to grow in patches of monolayer cells on the agarose pads for 24 h at 30 °C in the light. Before Brevisolum cells were added to initiate co-incubation.

In preparation for co-incubation with bacteria, Brevisolum cells were washed and concentrated 20-fold in fresh f/2 medium amended with 10 μg ml⁻¹ kanamycin. Prepared Brevisolum cells (10 μl) were spotted onto the middle of each agarose pad containing monolayer growth of R. pomeroyi. Co-incubation agarose pads were incubated in the dark, loosely covered with a plastic lid without contacting the agarose, for 24 h before imaging. Prior to imaging, a glass coverslip (60 mm × 24 mm; 0.17 ± 0.005 mm precision thickness; Carl Roth) was placed on the agarose pads carefully to avoid agitation of established phycospheres, and flipped onto the oil immersion objective for imaging. Only one tube of oil (24 h) was taken for each replicate, to avoid microscopy light-induced cell stress that could alter the phycosphere profile.

To calculate the spectral leakage correction matrix (Bbgamma; Supplementary Note 1), different colors of single-color constitutive control strains of R. pomeroyi (Supplementary Fig. 1) were grown on separate agarose pads for 24 h without phytoplankton, and imaged as described above.

Large-volume DMSP concentration measurement experiment. To estimate the DMSP concentration evolution in microfluidic observation chambers over time, a large-volume (8 ml) experiment was performed to allow sampling for DMSP concentration measurements (Supplementary Fig. 10). The experiment, while larger in volume by ~800 times compared to microfluidic experiments, preserved cell-to-volume ratio at all steps in the protocol. Three representative initial concentrations of DMSP were chosen, each of which was incubated in triplicates: 1 μM, 75 μM, and 1 mM. R. pomeroyi (strain Regular) was grown and prepared as described above, with modifications as described below. Three biological replicates (i.e., three different colonies as inocula) of overnight cultures were prepared in 2.5 ml of 1/2 YTSS rich medium per replicate. Overnight culture cells were washed and concentrated to 10 μg ml⁻¹ MBM. For each biological replicate, 153 μl of concentrated overnight culture was used to inoculate subculture flasks containing 115 ml of 10 mM glucose MBM amended with 25 μg ml⁻¹ kanamycin. This led to a cumulative dilution factor of 1/75 (vol/vol) of overnight culture for subculture preparation, consistent with microfluidic experiments. Subcultures were incubated in a shaken waterbath (200 rpm) for which ODmax was measured to be 0.02–0.04. A volume of 110 ml of subcultured cells per biological replicate was washed and concentrated fivefold in non-carbon amended MBM and 7.2 ml of this concentrated cells was allocated into each treatment flask of each biological replicate (150-ml glass Erlenmeyer flasks). The addition of 800 μl concentrated DMSP solution stocks (10x concentration, i.e., 10 μM, 750 μM, and 10 mM) and kanamycin (final concentration = 10 μg ml⁻¹) marked the initiation of incubation, with a starting volume of 8 ml. Final cumulative concentration from subcultured cells was 4.5× (nearly consistent with microfluidic experiments). One replicate of blank control flasks (i.e., without cells) representing each DMSP concentration condition was also prepared. All experimental flasks were sealed and incubated in the dark at room temperature (21±2.8 °C) and in the absence of agitation.

At each sampling time point, incubation flasks were swirled to resuspend sunken cells. From each flask, a 1.5-ml sample was taken for DMSP concentration measurement, and an additional 10-ml sample was placed in a microfluidic observation chamber for microscopy observation (imaged as described above). The first time point (0 h) was taken from the blank control flasks, for measurement of initial DMSP concentration. Subsequent time points, at which samples were taken from blank control flasks as well as from all replicate experimental conditions, were approximately 2.8, 8, and 24 h after the start of incubation with DMSP.

Each sample was immediately centrifuged at 2500 × g for 5 min to remove cells from solution. One milliliter of the supernatant was placed in an acid-washed 5-ml glass scintillation vial containing 3 ml methanol (99.9%, HPLC gradient grade; VWR International). Sample vials were sealed and stored in the dark at 4 °C until DMSP concentration measurement using ultra-high-performance liquid chromatography/high-resolution mass spectrometry (UHPLC/HRMS).

Chromatography/high-resolution mass spectrometry. To prepare samples for DMSP concentration measurements, 30 μl of each sample (processed and stored as described above) was diluted at 1/3 times with double distilled water (91.17% v/v), centrifuged (4500 × g, 5 min), and the supernatant was used for UHPLC/HRMS measurements. All UHPLC/HRMS results were obtained on a Dionex Ultima 3000 system (Thermo Scientific) coupled to a Q Exactive Plus Orbitrap mass spectrometer (Thermo Scientific).

UHPLC/HRMS quantification followed a previously reported protocol⁴⁹: the eluent consisted of high-purity water with 2% acetonitrile and 0.1% formic acid (solvent A) and 90% acetonitrile with 10% 5 mmol l⁻¹ aqueous ammonium acetate (solvent B). The flow rate was set to 0.60 ml min⁻¹. A linear gradient was used for separation with 100% solvent B (1 min), 20% B (6.5 min), 100% B (7.1 min), and 100% B (10 min) (to remove solvent). The LC separation column (SeQuant ZIC-HILIC guard column (5 μm, 2.1 × 15 mm)) equipped with a SeQuant ZIC-HILIC guard column (5 μm, 2.1 × 20 mm)) was kept at 25 °C. Electro spray ionization was performed in positive mode ionization, recording the mass range from 75 to 100 m/z, with the following parameters: capillary temperature 380 °C; spray voltage 3000 V; sheath gas flow 60 and auxiliary units at 50, and auxiliary gas flow 12 μl.
area [DMSP] = 470.54c [DMSP in nM] with r = 0.9999. Data analyses were performed using the software Thermo Xcalibur version 3.0.63.

Pre-exposure to sulfur experiment with Raman microspectroscopy. Raman microspectroscopy was utilized to infer uptake of DMSP at the single-cell level by measuring the deuterium-labeling status of cells incubated with deuterated DMSP ([\(^{2H}_3\)-DMSP]), in which the two CH groups of the DMSP molecule were labeled with deuterium in a protocol previously reported25 (Supplementary Fig. 11). Three incubation conditions were tested to probe the effect of sulfur satiation (due to pre-exposure of \(R. pomeroyi\) to 10 mM methionine) on DMSP uptake and cleavage pathway expression: \([^{2H}_3]\)-DMSP without pre-exposure to methionine; \([^{12}H]_3\)-DMSP with pre-exposure to methionine; and non-labeled DMSP without pre-exposure to methionine (negative control for Raman microspectroscopy signal).

The \(\text{P}10\text{m}/\text{mTFP}\) single-color \(R. pomeroyi\) reporter strain (cleavage pathway promoter-fusion with TFP) was used to avoid spectral interference with Raman microspectroscopy measurements (see below). An overnight culture in rich medium was prepared as described above. Two subculture conditions, with or without 10 mM l-methionine (Sigma-Aldrich), were prepared in 10 mM glucose MM amended with 25 μM 1k Kanamycin, and incubated for 4 h as described above. Subcultured cells were washed, concentrated threefold, and resuspended in the appropriate solution for incubation: 1 mM \([^{2H}_6]\)-DMSP MBM or 1 mM unlabeled DMSP MBM. All incubation conditions contained final concentrations of 1% methanol (sevent in which \([^{12}H]_3\)-DMSP was dissolved) and 10 μg/ml 1k Kanamycin. Treated cells were incubated at room temperature (22.8°C) in the dark for 5.5 h before imaging and Raman microspectroscopy measurements.

We utilized a commercial confocal Raman microspectroscopy (LabRAM HR Evolution; HORIBA Scientific) based on an inverted microscope (Eclipse Ti; Nikon) with two cameras: an sCMOS camera (ORCA-Flash 4.0; Hamamatsu) and a monochromatic Photonic filters installed to minimize interference with the Raman signal53. A thin liquid column containing cells was achieved by separating the sample in the bottom (CaF2) and top (glass, 18 mm × 18 mm, no. 1 thickness) coverslips with CaF2 surface interfacing with the 60× water-immersion objective (Plan Apo IR 63×1.20 NA; Nikon) with two cameras: an sCMOS camera (ORCA-Flash 4.0; Hamamatsu) and a monochromatic camera (#2) for positioning the laser. The microspectroscopy signal was acquired using the software Thermo Xcalibur version 3.0.63.

Data availability

The data that support the findings of this study are available from the corresponding authors on request (total data size approximately 1 TB). The source data underlying the Nature Research Reporting Summary linked to this article. All computer code (in MATLAB) developed for this study is available from the corresponding authors on request.

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Author contributions
J.R.S., J.-B.R., and R.S. designed the study. C.G. constructed the reporter strains and performed the microfluidic and agarose pad experiments. C.G., and K.S.L. performed the Raman microscopy experiments. C.G., S.F., and G.P. performed the large-volume DMSP concentration measurement experiment. C.G., V.I.F., and R.S. created the image analysis software. C.G., V.I.F., J.R.S., J.-B.R., and R.S. wrote the paper. All authors edited the paper before submission.

Competing interests
The authors declare no competing interests.

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