1 **Bacteria are important dimethylsulfoniopropionate producers in coastal sediments**

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18 **Dimethylsulfoniopropionate (DMSP) and its catabolite dimethyl sulfide (DMS) are key**
19 **marine nutrients[1,2], with roles in global sulfur cycling[2], atmospheric chemistry[3],**
20 **signalling[4,5] and, potentially, climate regulation[6,7]. DMSP production was previously**
21 **thought to be an oxic and photic process, mainly confined to the surface oceans.**

22 **However, here we show that DMSP concentrations and DMSP/DMS synthesis rates**
23 **were higher in surface marine sediment from e.g., saltmarsh ponds, estuaries and the**
24 **deep ocean than in the overlying seawater. A quarter of bacterial strains isolated from**
25 **saltmarsh sediment produced DMSP (up to 73 mM), and previously unknown DMSP-**
26 **producers were identified. Most DMSP-producing isolates contained *dsyB*[8], but some**
27 **alphaproteobacteria, gammaproteobacteria and actinobacteria utilised a methionine**
28 **methylation pathway independent of DsyB, previously only associated with higher**
29 **plants. These bacteria contained a methionine methyltransferase '*mmtN*' gene - a**
30 **marker for bacterial DMSP synthesis via this pathway. DMSP-producing bacteria and**
31 **their *dsyB* and/or *mmtN* transcripts were present in all tested seawater samples and**
32 ***Tara* Oceans bacterioplankton datasets, but were far more abundant in marine surface**
33 **sediment. Approximately 10^8 bacteria per gram of surface marine sediment are**
34 **predicted to produce DMSP, and their contribution to this process should be included**
35 **in future models of global DMSP production. We propose that coastal and marine**
36 **sediments, which cover a large part of the Earth's surface, are environments with high**
37 **DMSP and DMS productivity, and that bacteria are important producers within them.**

38 Approximately eight billion tonnes of DMSP is produced by phytoplankton in the Earth's
39 surface oceans annually[9]. However, surface sediment from saltmarsh ponds, an estuary and
40 the deep ocean (with high pressures and no light) contained DMSP levels (5-128 nmol DMSP
41 g^{-1}) that were up to ~three orders of magnitude higher than the overlying seawater (0.01-0.70
42 nmol DMSP ml^{-1}) (Fig. 1a-b, Supplementary Tables 1a and 2), a phenomenon also observed
43 in **10,11**. DMSP concentration decreased with depth, being much lower in anoxic sediment,
44 but even in deeper sediments the concentration was approximately an order of magnitude
45 higher than in the overlying seawater (Supplementary Table 1a). This study focused on
46 DMSP synthesis in coastal surface sediments, where DMSP concentrations were highest. The

47 DMSP-producing cordgrass *Spartina* is proposed to be the major DMSP and DMS source in
48 many saltmarshes[12,13]. Indeed, high DMSP levels were found in *Spartina anglica* roots and
49 leaves around the sampled ponds, and the highest sediment DMSP levels detected were
50 adjacent to this cordgrass (Supplementary Fig. 1a-b). However, *S. anglica* rhizosphere and
51 phyllosphere samples contained bacteria with the genetic potential to synthesise DMSP
52 (Supplementary Table 3), and we cannot dismiss the possible contribution of bacteria to
53 DMSP levels in *S. anglica* and/or the surrounding environment. Furthermore, surface
54 sediment DMSP concentrations stabilise ~20 cm away from the *Spartina* (Supplementary
55 Fig. 1b). Yarmouth estuary, which also had high DMSP levels (Supplementary Table 1a),
56 lacked *Spartina* and was populated with *Aster tripolium*, a halophyte not known to
57 accumulate DMSP, but which contained DMSP, at lower levels than *S. anglica*
58 (Supplementary Fig. 1a). As with DMSP standing-stock concentrations, DMSP and DMS
59 synthesis rates were much higher in surface sediment than the overlying water samples
60 (Table 1, Supplementary Fig. 2). These data suggest that a sizeable amount of DMSP in the
61 sediment may result from microbial biosynthesis, rather than solely from sinking particles or
62 DMSP-producing plants. We propose that surface coastal and marine sediments in general,
63 which cover >70% of the Earth's surface[14], are highly active environments for microbial
64 DMSP biosynthesis and catabolism, generating the climate-active gas DMS.

65 Microbial community analysis was performed on Stiffkey saltmarsh surface sediment to
66 identify potential DMSP-producers. This was dominated by bacteria (~91% of 16S rRNA
67 gene sequences {Supplementary Fig. 4}), of which ~2.3 ±0.6% belonged to genera that
68 include *dsyB*-containing species, a reporter gene for bacterial DMSP synthesis[8]
69 (Supplementary Figs. 5 and 6, Supplementary Tables 4 and 5). Furthermore, metagenomic
70 analysis predicted that ~1% of bacteria contain *dsyB*, spanning functional
71 methylthiohydroxybutyrate (MTHB) methyltransferases[8] (Supplementary Fig. 7). This

72 abundance was higher than most DMSP lyases (enzymes that cleave DMSP, releasing DMS)
73 apart from the genes encoding DddD, DddL and DddP (present in 1.1, 4.8 and 6.6% of
74 bacteria, respectively) (Supplementary Table 7), which are likely important DMS-producing
75 enzymes in these sediments. Eukaryotic plastid 16S rRNA genes, predominately from
76 diatoms, represented ~9% of the community sequences (Supplementary Fig. 4).
77 *Asterionellopsis*, a member of the Fragilariophyceae family with low or undetectable
78 intracellular DMSP levels[15], was the most abundant diatom (~6% of 16S rRNA gene
79 community data {Supplementary Fig. 5}). A 3 μm diameter chain-forming *Asterionellopsis*
80 *glacialis* (strain PR1) isolated from Stiffkey sediment, with 99% 16S rRNA gene identity to
81 the dominant *Asterionellopsis* in the amplicon data, produced low intracellular DMSP levels
82 (0.21 mM) (Supplementary Fig. 8). No DMSP synthesis genes have been identified in the
83 Fragilariophyceae family of diatoms, but in Stiffkey metagenomes the eukaryotic DMSP
84 synthesis gene *DSYB*[16] was ~13-fold less abundant than *dsyB* (Supplementary Table 7). The
85 plastid 16S rRNA gene sequences of other DMSP-producing eukaryotes were detected at
86 very low levels, including *Phaeodactylum* (0.4%) and *Thalassiosira* (0.3%). Given the
87 abundance of DMSP-producing diatoms and bacteria in Stiffkey sediments, both are likely
88 important DMSP producers in such photic marine environments.

89 Incubation experiments were conducted to enrich for and isolate DMSP-producing bacteria
90 from Stiffkey sediment. DMSP production in 'enriched' sediment slurries was enhanced by
91 incubation in enrichment media with increased salinity, MTHB addition, and reduced
92 nitrogen levels (conditions enhancing DMSP synthesis in *Labrenzia*[14]). Over 14 days DMSP
93 levels were consistently highest (day 4 onwards) in microbial particulates from enriched
94 samples (Supplementary Fig. 9). The proportion of DMSP-producing bacterial isolates
95 (Supplementary Table 8) in the enriched sediment increased to 71%, from 25% in natural

96 sediment. This supports these incubation experiments as an effective enrichment
97 methodology for DMSP-producing bacteria.

98 Enriched and control microbial community profiles greatly differed from those in natural
99 sediment, likely due to the addition of media, mixed carbon source and/or other differences
100 from the natural conditions (temperature, dissolved oxygen, etc) during the incubation
101 (Supplementary Figs. 4, 5, 7 and 10). Importantly, the enriched microbial community had
102 several clear genus-level differences to the natural and control samples. The abundance of
103 known DMSP-producers *Oceanicola* (2.1 ±0.01%) and *Ruegeria* (4.5 ±0.1%) significantly
104 increased in the enriched samples (Supplementary Figs. 5 and 10), alongside genera of
105 DMSP-producing alpha- and gammaproteobacterial isolates from this study, e.g.,
106 *Marinobacter* (3.2 ±0.4%), *Novosphingobium* (4.7 ±0.9%) and *Alteromonas* (20.7 ±2.4%).
107 Bacteria of the latter three genera, comprising ~0.6% of the natural sediment community and
108 lacking *dsyB* in their available genomes, likely contributed to the enhanced DMSP levels seen
109 in enriched samples (Supplementary Fig. 9). The abundance of *dsyB*-containing bacterial
110 genera (11.7 and 10.5%, Supplementary Table 5), the *dsyB* gene (determined by qPCR and
111 metagenomics {Fig. 1c, Supplementary Table 7}), and *dsyB* transcripts (Fig. 1c) showed no
112 significant differences between control and enriched samples. However, the DsyB diversity
113 varied somewhat between the two (Supplementary Fig. 6). It is possible that bacteria with the
114 more abundant DsyB variants in the enriched samples may contribute to the higher observed
115 DMSP levels, e.g. by producing higher intracellular DMSP concentrations. Alternatively,
116 there may be additional unknown DMSP synthesis genes/pathways contributing to the
117 enhanced DMSP levels seen.

118 *Novosphingobium* sp. BW1 was used to investigate *dsyB*-independent DMSP production
119 pathways. Of the known DMSP synthesis pathway intermediates (Fig. 2a)[17], BW1 DMSP

120 production was significantly enhanced by adding methionine (Met), the universal DMSP
121 precursor, and *S*-methyl-methionine (SMM), a common plant metabolite[18,19] and intermediate
122 of the methylation pathway in DMSP-producing plants, e.g., *Spartina*[20] (Fig. 2b). Met (0.90
123 ±0.01 mM) and another intermediate in this pathway, DMSP-amine (0.13 ±0.02 mM), were
124 detected in BW1 cell extracts by HPLC (Supplementary Fig. 11) and SMM was detected by
125 LC-MS, further supporting the methylation pathway as the likely BW1 DMSP synthesis
126 pathway. DMSP-amine addition did not enhance DMSP production, possibly due to the
127 ability of BW1 to import DMSP-amine, or because DMSP-amine may not induce the
128 expression of DMSP synthesis genes. BW1 cell extracts had *S*-adenosyl-Met (SAM)-
129 dependent Met methyltransferase (MMT) activity, converting Met to SMM (3.6 µmol min^{-1}
130 µg $protein^{-1}$). Although some bacteria catabolise SMM[19,21] for use as a methyl donor, none
131 have previously been shown to possess MMT activity. Addition of 4-methylthio-2-
132 oxobutyrate (MTOB) also enhanced BW1 DMSP production (2.5-fold), but to a lesser extent
133 than Met or SMM (7- and 13-fold, respectively), perhaps indicating that BW1 has an active
134 Met salvage pathway generating Met from MTOB[22].

135 By screening a BW1 genomic library, a gene conferring MMT activity (EC2.1.1.12) termed
136 *mmtN* was identified (Supplementary Table 8, Supplementary Fig. 12). Purified MmtN had
137 SAM-dependent MMT activity, but did not methylate related compounds, including MMPA,
138 glycine and MTHB (Supplementary Figs. 13a and 14). MmtN homologues (≥ 54% aa
139 identity), exist in many marine alphaproteobacteria, one gammaproteobacterium and some
140 actinobacteria, representatives of which produced DMSP, with *mmtN*-like genes that were
141 cloned and functionally ratified (Fig. 3, Supplementary Fig. 12, Supplementary Table 8). A
142 recent biochemical study characterised MmtN from *S. mobaraensis* and *Rhodovulum* sp. P5
143 as having MMT activity[23], with K_M values comparable to those reported here for
144 *Novosphingobium* MmtN (Supplementary Fig. 14).

145 Thus, *mmtN*, like *dsyB*[8] and *DSYB*[16], is another robust reporter gene for an organism's
146 potential to synthesise DMSP. *mmtN*-containing bacteria were less abundant than those with
147 *dsyB* in tested seawater and sediment samples (Fig. 1c, Supplementary Tables 5 and 7).
148 However, the abundance of *mmtN*-containing bacteria was higher in the enriched versus
149 control incubation samples, suggesting that MmtN-dependent DMSP production may be a
150 significant contributor to the increased DMSP levels seen under the enrichment conditions
151 (Fig. 1c, Supplementary Tables 5 and 7).

152 The *mmtN* gene is required for DMSP synthesis in *T. profundimaris,* since an *mmtN*$^-$ mutant
153 did not produce DMSP, and was restored by complementation with cloned *mmtN* (Fig. 2c,
154 Supplementary Table 8, Supplementary Fig. 13b). Further work is required to elucidate the
155 complete MmtN-dependent DMSP synthesis pathway, which likely involves a suite of genes
156 (two distinct types) encoding a putative aminotransferase, dehydrogenase and decarboxylase
157 adjacent to *mmtN* in many marine bacterial genomes (Supplementary Fig. 12). Liao and
158 Seebeck[23] found that *S. mobaraensis* candidate gene products from one such suite of genes
159 (SMM decarboxylase, DMSP-amine aminotransferase and DMSP-aldehyde dehydrogenase
160 {Figure 2a}) had the expected enzyme activities. We also show that mutation of the putative
161 DMSP-amine aminotransferase (TH2_03140), part of the second suite of genes
162 (Supplementary Fig. 12) in *T. profundimaris,* caused a 73% reduction in DMSP compared to
163 wild type *T. profundimaris.* This suggests that at least one of these linked genes encodes a
164 downstream enzyme in the DMSP biosynthesis pathway in *T. profundimaris*. The *mmtN*$^-$
165 mutant displayed no significant growth reduction or competitive disadvantage compared to
166 the wild type strain in response to increased salinity and/or reduced nitrogen conditions,
167 which were known to enhance DMSP production in this bacterium (Supplementary Fig. 15).
168 Similar results were found with a *Labrenzia dsyB*$^-$ mutant[8], which, like *T. profundimaris,* also
169 produces the nitrogenous osmolyte glycine betaine (GBT). Indeed, the *T. profundimaris*

170 *mmtN*$^{-}$ mutant displayed enhanced GBT production levels compared to the wild type,
171 suggesting that GBT, and/or other osmolytes produced by these bacteria, compensate for the
172 loss of DMSP (Fig. 2c, Supplementary Fig. 13b).

173 MmtN proteins form a distinct group (Fig. 3), but have ≤30% identity to the N-terminal
174 methyltransferase domain of distantly related and larger (33 versus 115 kDa) plant Met *S*-
175 methyltransferase MMT enzymes (PLN02672) (Fig. 3). These contain an extra C-terminal
176 aminotransferase domain (pfam00155) thought to have a regulatory role[24]. The amino acid
177 and domain differences between the bacterial MmtN and plant MMT enzymes are likely
178 responsible for the ~10-fold higher K_M values observed for the former[24]. Genes encoding full-
179 length plant-like MMT enzymes exist in some bacterial genomes, mainly deltaproteobacteria
180 (Fig. 3), and four such bacteria were tested for DMSP production. Only *Pseudobacteriovorax*
181 *antillogorgiicola* DSM103413 produced DMSP, at low levels (Supplementary Table 8).
182 Thus, unlike *mmtN*, the presence of the full-length plant-like MMT in an organism is not a
183 good indicator of DMSP production. Within the group containing functional MmtN proteins,
184 we did not find monophyly among the major bacterial groups, suggesting that *mmtN* may
185 have transferred between bacteria by horizontal gene transfer. The high level of sequence
186 divergence between bacterial *mmtN* and full-length MMT genes suggests that this pathway is
187 ancient, arising independently in bacteria and plants, or possibly through ancient horizontal
188 gene transfer.

189 DMSP-producing bacteria (containing DsyB and/or MmtN) are predicted by qPCR to
190 constitute 0.1-3.6% of bacteria in the tested marine sediment samples, from saltmarsh ponds,
191 an estuary and the deep ocean (Supplementary Table 9). Indeed, the percentage of DMSP-
192 producers predicted by metagenomic analysis is ~1.1% (Supplementary Table 7), which,
193 when applied to the estimated 1.99×10^{10} bacterial cells g sediment^{-1} in Stiffkey surface

194 sediment (Supplementary Table 10) suggests an abundance of ~10^8 DMSP-producing
195 bacteria g sediment^{-1}, with intracellular DMSP levels ranging from 0.66–73 mM (Fig. 1c,
196 Supplementary Tables 7 and 9). DMSP-producing bacteria were much less abundant in the
197 ocean microbial reference gene catalogue metagenomic database (OM-GRC)[25]
198 (predominantly surface seawater samples) and in tested coastal seawater samples compared
199 to the surface sediment, but they still represent 0.3-0.6% of a reported 10^6 bacteria ml^{-1} in
200 seawater[26] (Fig. 1c, Supplementary Tables 7 and 9). These predictions are likely
201 underestimations, since some isolated bacteria lacking *dsyB* and *mmtN* were shown to
202 produce DMSP (e.g., *Marinobacter,* representing ~0.5% of the natural sediment community),
203 probably via unidentified DMSP synthesis genes/pathways. The *dsyB* gene was transcribed in
204 all tested samples, but was > three orders of magnitude higher, per unit mass, in surface
205 sediment than in pond water and surface seawater (Fig. 1c). Furthermore, *dsyB*[16] and *mmtN*
206 transcripts are omnipresent or mostly present, respectively, at varied levels in *Tara* Oceans
207 bacterioplankton metatranscriptome databases (Supplementary Tables 11 and 12). In
208 seawater incubation experiments *Novosphingobium* sp. BW1 (*mmtN*$^+$), *Pelagibaca*
209 *bermudensis* (*dsyB*$^+$) and *Labrenzia* LZB033 (*dsyB*$^+$) produced DMSP and contributed to the
210 dissolved pool, demonstrating activity under closer to natural conditions (Supplementary Fig.
211 16, Supplementary Table 13). These data are consistent with a large global biomass of
212 DMSP-producing bacteria actively synthesising DMSP in marine sediment and seawater
213 environments. This work shows that bacteria likely contribute to DMSP levels in seawater
214 environments, but further work is required to evaluate their significance. Additionally, the
215 contribution of bacteria, and in some cases that of benthic algae, to total DMSP levels is
216 likely to be far higher in marine surface sediments, which per unit mass are more productive
217 than the overlying seawater. Moreover, while the DMSP content in the anoxic saltmarsh
218 sediment is far lower than the oxic surface layer (Supplementary Table 1a), it is still ~5- to

219 10-fold higher than that of the overlying seawater, and is an environment in which bacterial
220 DMSP production is unstudied. This study challenges the notion that DMSP production is
221 mainly an oxic and photic process and suggests that global models for DMSP and DMS
222 production should consider marine surface sediments and bacteria as important contributors.

223 **Methods**

224 **General Scientific Practices**

225 **Chemical syntheses**

226 DMSP was synthesised from DMS (Sigma-Aldrich) and acrylic acid (Sigma-Aldrich) as
227 described in Todd *et al.*[27]. DMSHB, DMSP-amine and SMM were synthesised as in Curson
228 *et al.*[8]. Met, MTOB, MTHB and MTPA are commercially available and were obtained from
229 Sigma-Aldrich.

230 **Quantification of DMS/DMSP/SMM by GC**

231 All gas chromatography (GC) assays involved measurement of headspace DMS, either
232 directly produced or via alkaline lysis of DMSP or SMM, using a flame photometric detector
233 (Agilent 7890A GC fitted with a 7693 autosampler) and a HP-INNOWax 30 m × 0.320 mm
234 capillary column (Agilent Technologies J&W Scientific). Unless otherwise stated, all
235 DMSP/SMM GC measurements were performed using 2 ml glass vials containing 0.3 ml
236 liquid samples and sealed with PTFE/rubber crimp caps. To quantify DMSP, 0.2 ml of
237 overnight culture was added to a 2 ml vial, 0.1 ml 10 M NaOH was added, vials were
238 crimped immediately, incubated at 22°C overnight in the dark and monitored by GC. To
239 quantify SMM production, 0.2 ml of culture and 0.1 ml of 10 M NaOH were sealed in glass
240 vials and heated at 80°C for 10 min before incubating overnight and sampling. An eight-point
241 calibration curve was produced by alkaline lysis of DMSP and SMM standards in water. The
242 detection limit (per 300 µl sample in 2 ml GC vial) was 0.015 nmol for DMSP and 1.5 nmol
243 for SMM.

244 **Detection of DMSP, GBT and SMM by LC-MS**

245 LC-MS was used to confirm that bacteria were producing DMSP and at similar levels to
246 those determined by GC, ruling out the possibility that DMS detected by GC was due to some
247 other compound and not DMSP. The method used for the detection of DMSP and GBT was
248 as described in Curson *et al.*[8]. SMM detection followed the same method. All samples (15 µl)
249 were analysed immediately after being extracted. The targeted mass transition corresponded
250 to $[M+H]^+$ of DMSP (*m/z* 135), GBT (*m/z* 118) and SMM (*m/z* 165) in positive mode. To
251 confirm the presence of the compounds in the biological samples, standards were also run
252 (10-50 µM).

253 **Quantification of Met, DMSP and SMM by HPLC**

254 HPLC methods were developed to determine Met, SMM and DMSP-amine as fluorescent
255 adducts after pre-column derivatisation with ortho-phthaldialdehye (OPDA)[28], but employing
256 mercaptoethanol, instead of mercaptopropionic acid, as the thiol reagent. Samples (50 µl)
257 were mixed with 50 µl derivatisation reagent (5 mg OPDA in 5 ml methanol, buffered with
258 35 ml 1 M potassium borate buffer, pH 10.4, mixed with 84 µl mercaptoethanol), and reacted
259 for 3 min before injecting a 10 µl sample onto a 4.6 x 250 mm Synergi Hydro-RP
260 (Phenomenex) column, eluted according to Caddick *et al* [28]. Fluorescent adducts were
261 detected with a Jasco FP-920 fluorescence detector set at Ex 332 nm, Em 445 nm, with
262 bandpass 18 nm and gain 10. Met, SMM and DMSP-amine standards yielded correlation
263 coefficients of >0.999, >0.999 and >0.995, respectively, for 5-point calibration in the range 1-
264 20 µM. A 5-point calibration for Met in the range 0.2-2 µM in seawater media yielded a
265 correlation coefficient >0.984. The limit of detection of Met, at 3x noise, of the
266 chromatogram, was estimated to be c. 0.02 µM in samples. Seawater was filtered with a 0.45
267 µm syringe filter. For detecting dissolved Met in the sediment, 0.3 g sediment was diluted

268 with 3 ml ESAW artificial seawater medium then centrifuged at 500 *g* for 20 minutes. The
269 supernatant was removed and filtered with a 0.45 µm syringe filter before analysing for Met.

270 **Quantification of DMSP by purge trap**

271 Total DMSP samples of seawater and sediment were fixed with 50% (v/v) H_2SO_4 and stored
272 at room temperature for 2 days. For seawater samples, 250 µl 50% H_2SO_4 was directly added
273 to 25 ml of seawater and then sealed. For sediment samples, 0.5 g of sediment was first
274 mixed with 25 ml distilled water, then added to 250 µl 50% H_2SO_4 and sealed. For analysis, 1
275 ml of 10 M NaOH was injected into 5 ml of the preserved seawater sample and then sealed
276 and incubated in the dark at 22°C for 16 h. To measure DMSP in sediment, the samples were
277 centrifuged at 5,000 *g* and 5 ml of the supernatant of the preserved mix was used. The
278 liberated DMS was measured using the purge and trap method[29]. Briefly, sulfur gases were
279 sparged from the sample with nitrogen and trapped in a loop of tubing immersed in liquid
280 nitrogen. The trapped gases were desorbed with hot water (above 90°C) and analysed by GC.

281 The DMSP content of seawater was determined by taking 25 ml seawater mixed with H_2SO_4
282 (to 0.5%). This mix was incubated at room temperature for 2 days and 5 ml was then mixed
283 with 1 ml 10 M NaOH and incubated at 22°C for 16 h in the dark, before using a modified
284 purge and trap method as described in Zhang *et al.*[30] to collect the DMS released by the
285 sample. The samples were purged for 20 mins and then compounds were detected by GC.

286 **<u>Site Characterisation</u>**

287 **Environmental parameters of Stiffkey saltmarsh**

288 The oxygen saturation was measured at the water surface, half depth (80 mm) and above the
289 water/sediment interface (160 mm) using a Jenway 970 and a 2-point calibration with filtered

290 seawater in equilibrium with air (100% oxygen saturation) and a 2 M sodium sulfite solution
291 (0% oxygen saturation). Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN)
292 measurements were made using triplicate measurements by a Skalar Formacs CA15 analyser,
293 employing a six-point calibration. TDN represents the sum of all dissolved nitrogen-
294 containing species (excluding dinitrogen {N_2}) and includes organic nitrogen species as well
295 as nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and nitrous oxide (N_2O).

296 Nutrient analysis was performed using a Seal AA3 AutoAnalzyer at CEFAS, Lowestoft.
297 Phosphate was measured as described in Murphy and Riley[31], ammonium as in Jones[32] and
298 nitrate and nitrite as in Armstrong *et al*.[33].

299 **Sampling sediment**

300 The majority of the enrichment and isolation work described in this study was performed on
301 surface sediment samples from Stiffkey saltmarsh, UK (52.9643, 0.9255) (Supplementary
302 Table 1a-b). Triplicate marine sediment samples were collected using sterile acrylic corers at
303 least 40 cm from the banks of the ponds. DMSP content was measured in samples taken from
304 the overlying water (200 µl), which was ~15-20 cm deep, from the surface sediment layer
305 (top 1 cm) and from three anoxic depths (5 cm, 10 cm and 15 cm). Cores were transported
306 immediately to the laboratory and processed on arrival. Surface sediment and water (~20-30
307 cm deep) from Cley saltmarsh, UK (52.9586, 1.0473) and Yarmouth Estuary, UK (52.6133,
308 1.7162) were also sampled for comparison of DMSP production rates as well as expression of
309 key genes involved in DMSP synthesis. Finally, the R/V Dong Fang Hong 2 cruise
310 (September 2016) sampled surface seawater and deep-sea surface sediment (4,500 m depth)
311 from the Mariana Trench (10.4091, 142.3569) using a box corer.

312 **DMSP/DMS rate experiments with ^{3}H-methionine**

313 Experiments to establish rates of DMS/DMSP production in surface sediment from Stiffkey,
314 Yarmouth and Cley were undertaken as follows. For seawater samples, 10 ml seawater was
315 added to a 30 ml universal bottle. For sediment samples, 1 g sediment and 10 ml autoclaved
316 seawater was added to a 30 ml universal bottle. Autoclaved sediment and seawater were used
317 as negative controls. L-[methyl-^{3}H]-methionine (85 Ci mmol^{-1}; Perkin Elmer) was added to a
318 final concentration of 6 nM (185 kBq) and samples were incubated at 22°C for the times
319 specified. For DMSP measurements, at each timepoint (T=30, 60, 90, 180, and 240 min) 1 ml
320 of seawater or sediment/seawater slurry were removed to a new 30 ml universal bottle
321 containing 13 µl of 20% (v/v) H_2SO_4 (to prevent further bacterial activity, stabilise the DMSP
322 and convert ^{3}H volatiles to non-volatiles) and mixed. A 1.5 ml centrifuge tube containing a
323 DMS trap was placed in the top of the universal bottle. The DMS trap consisted of half a 25
324 mm GF/F glass microfibre filter folded and soaked in 100 µl 3% (v/v) hydrogen peroxide to
325 collect headspace DMS, as in Slezak *et al.*[34]. To release the ^{3}H-DMS from any ^{3}H-DMSP
326 present, 1 ml of 10 M NaOH was added to the seawater/slurry in the universal bottle
327 containing the trap. Universal bottles were sealed, shaken gently and incubated at 22°C for 24
328 h to trap ^{3}H-DMS. Filters were removed to a 20 ml polyethylene vial containing 4 ml
329 scintillant (Ecoscint A) and the vials mixed. Samples were left in the dark for 1 h before
330 scintillation counting on a Hidex 300 SL scintillation counter. DMS measurements were done
331 in the same way as described for DMSP except that the DMS trap was placed directly into the
332 universal bottle containing the seawater or sediment slurry without added NaOH. This
333 allowed DMS produced and released into the headspace, through microbial cleavage of any
334 ^{3}H-DMSP, to be captured in the trap. Filters were removed after 24 h and ^{3}H measured as for
335 the DMSP samples. Counts per minute values recorded were used to calculate the rate of
336 DMSP/DMS production expressed as fmol g^{-1} min^{-1} or fmol ml^{-1} min^{-1} for sediment or

337 seawater samples respectively and DMS production in sediment over a 24 h period expressed
338 as nmol g^{-1}.

339 Rates of DMSP or DMS production were calculated based on the amount of labelled product
340 produced (as ^{3}H-DMS). For DMSP production rates in sediment, experiments were done with
341 the labelled ^{3}H-Met substrate in tracer amounts (< 0.6%) relative to the dissolved ambient
342 Met concentration, estimated here to be 3.94 ±0.89 µM for Stiffkey, 2.71 ±0.20 µM for Cley,
343 and 1.04 ±0.88 µM for Yarmouth[35]. The rate derived from the labelled product was then
344 multiplied according to the factor of dissolved ambient methionine concentration relative to
345 the added labelled ^{3}H-Met concentration (6 nM). For DMSP production rates measured in
346 Stiffkey seawater, dissolved ambient Met was 0.34 ±0.06 µM, and calculations were made as
347 for DMSP in sediment above to correct by the factor of dissolved ambient methionine relative
348 to labelled ^{3}H-Met added. This value for dissolved ambient Met in seawater was used for all
349 sites. The values were converted to pmol DMSP m^{-2} h^{-1} and pmol DMSP cm^{-3} h^{-1} for
350 sediment and seawater respectively by normalising wet to dry sediment using a factor of 0.5 g
351 cm^{-3}, determined in weight/drying measurements[36] on sediments comparable to those of
352 Stiffkey. Finally, it was assumed that this type of active, oxic sediment makes up the top 1 cm
353 of sediment, converting rates cm^{-3} to rates m^{-2}.

354 For measurements of DMS produced from dissolved ambient Met over 24 hours in sediment,
355 these values were calculated from the labelled ^{3}H-DMS produced. As with experiments for
356 DMSP production rates in sediment described above, labelled ^{3}H-Met was used as substrate
357 in tracer amounts (< 0.6%). The amount of labelled ^{3}H-DMS produced was corrected by the
358 factor of the dissolved ambient methionine concentration in sediment at each location (see
359 above) relative to the added labelled ^{3}H-DMSP concentration (6 nM).

360 These DMSP and DMS production rate estimations are performed under lab conditions that
361 do not consider the ambient Met already within cells, thus, we advise caution in their
362 extrapolation beyond this level.

363 **DMSP cleavage rate experiments**

364 Experiments to approximate the rate of DMSP catabolism generating DMS in surface
365 sediment and overlying pond or seawater from Stiffkey, Yarmouth and Cley were performed
366 as follows. For pond or seawater samples, triplicate 10 ml samples were added to a 140 ml
367 serum vial. For sediment samples, 1 g sediment and 10 ml autoclaved seawater was added to
368 a 140 ml serum vial in triplicate. DMSP was added to a final concentration of 0.1 mM
369 alongside controls with no DMSP, and vials were crimp-sealed immediately. DMS headspace
370 concentrations were measured at T=0, 30, 60, 90 and 120 min by GC (see above) using
371 manual injections. These measurements were used to calculate the rate of DMS production,
372 expressed as nmol g^{-1} min^{-1} or nmol ml^{-1} min^{-1} for sediment or seawater samples respectively.

373 **DMSP analysis on *Spartina anglica,* the surrounding surface sediment and *Aster***
374 ***tripolium***

375 Plant and sediment samples were taken during low tide from ponds in Stiffkey and Cley
376 saltmarsh and from Yarmouth estuary. *A. tripolium* and *S. anglica* plants were carefully
377 uprooted and placed in sterile plastic bags. Surface sediment from Stiffkey was sampled as
378 above, following a 100 cm transect moving away from *Spartina,* sampling every 10 cm. Plant
379 material was washed to remove sediment and separated into different tissues (roots &
380 shoots/leaves for *S. anglica* and stems and leaves for *A. tripolium*) using ethanol sterilised
381 scissors and tweezers. The phyllosphere and rhizosphere of *S. anglica* were sampled by
382 washing 10 g leaves and 5 g roots in 10 ml sterile water with vortexing for 5 min, and

383 repeating five times. The five washates were centrifuged for 10 min at 15,000 *g* and DNA
384 was extracted from the pellets. This DNA was used as a template for qPCR analysis to test
385 for the presence and abundance of *dsyB* and *mmtN*, with the values from each of the five
386 washates being pooled to give total phyllosphere and rhizosphere gene abundance,
387 normalised to the weight of plant tissue washed (Supplementary Table 3).

388 Between 1-5 g (fresh weight) of tissue was ground to fine powder particles with liquid
389 nitrogen using a pre-cooled sterile ceramic mortar and pestle. To measure DMSP content,
390 approximately 0.1 g (fresh weight) of the ground material was added to 2 ml glass GC vials
391 and 300 µl 10 M NaOH was immediately added and vials were sealed with 11 mm crimp
392 caps with rubber/PTFE septa and mixed. For the transect samples, 10-20 g of sediment was
393 mixed thoroughly to ensure a homogenous sample. Replicates of ~0.1 g (wet weight) of this
394 mix were weighed into GC vials and mixed with 300 µl 10 M NaOH before crimp-sealing, as
395 above. Samples were left overnight in the dark at 22°C before GC analysis (see
396 'Quantification of DMS/DMSP/SMM by gas chromatography').

397 **Isolation of *Asterionellopsis glacialis***

398 In order to isolate epipelic diatoms present on the surface of saltmarsh pond sediment,
399 samples were taken by scraping the top 0.5-1 cm surface layer of the sediment. These were
400 then subsampled and inoculated into 250 ml flasks containing F/2 medium (made with 0.2
401 µm-filtered sterile Stiffkey pond water, 32 practical salinity units {PSU}); Guillard and
402 Ryther [37]). Several monoclonal isolates of pennate diatoms, including *Asterionellopsis*, were
403 established using the single-colony isolation technique described in Andersen *et al*.[38]. Isolates
404 were allowed to grow for 2-3 weeks at a constant temperature of 22°C under a 12:12h
405 light:dark photoperiod with a constant photon flux of 120 µE $m^{-2}s^{-1}$ (QSL-100 Quantum

406 Scalar Irradiance Meter, Biospherical Instruments, San Diego, USA) provided by Philips
407 MASTER TL-D 58W/840 white tubes. Isolates from enriched cultures were then further
408 purified and unnecessary contaminating picoplankton were removed by dilution. Once
409 purified, strains were transferred to 42-well plates and allowed to grow for approximately 2-3
410 weeks. Cultures were treated with multiple rounds of antibiotic treatment (400 $\mu g\ ml^{-1}$
411 streptomycin, 50 $\mu g\ ml^{-1}$ chloramphenicol, 20 $\mu g\ ml^{-1}$ gentamicin and 100 $\mu g\ ml^{-1}$ ampicillin)
412 to remove as many bacteria as possible. Clonal cultures were then transferred and up-scaled
413 to culture flasks (Nunc™ EasYFlask with Filter Caps, 75 cm^2 cell culture area, Thermo
414 Fisher Scientific) containing 20 – 40 ml F/2 medium (0.2 μm-filtered sterile 50:50 pond
415 water and ESAW artificial seawater, 35 PSU). The isolate used in this study was a strain
416 termed *Asterionellopsis glacialis* strain PR1. For culturing *A. glacialis* PR1 for DMSP
417 quantification, 30 ml of stock culture (3×10^5 cells ml^{-1}) was inoculated to 200 ml F/2
418 medium (made with ESAW artificial seawater medium, 35 PSU) in triplicate. Growth was
419 monitored every day by cell counting with a Zeiss Primovert inverted optical microscope and
420 a Sedgewick-Rafter counting cell. Cells were harvested after 24 days (in stationary phase,
421 Supplementary Fig. 8) and assayed for DMSP as in Curson *et al.*[16]. The cell volume of *A.*
422 *glacialis* PR1 used for intracellular DMSP calculations was 654 μm^3 and this was based on
423 calculations as in Naz *et al.*[39].

424 **DNA extraction and PCR amplification of rRNA genes from *A. glacialis* PR1**

425 PR1 cells were harvested by centrifuging 100 ml of culture containing 3.34×10^5 cells ml^{-1} for
426 10 minutes at 5,000 *g* and genomic DNA was extracted as described in Yin *et al.*[40], with the
427 following modifications. Cell disruption was achieved through bead beating at 6 m s^{-1} for 60
428 s with a Bead blaster 24 bead beater (Benchmark, Edison, NJ, USA), using silica beads
429 (Lysing Matrix E, MP Biomedicals, Cambridge, UK) in 60 μl of 10% (w/v) sodium dodecyl

430 sulfate (SDS). After cell disruption, 6 μl of proteinase K (10 mg ml^{-1}) was added to the
431 sample and incubated for 20 mins at 65°C, then centrifuged at 15,000 *g* for 10 mins. Nucleic
432 acid extracts were precipitated in an equal volume of cold isopropanol and washed with 800
433 μl cold 75% ethanol, and the pellets dissolved in 100 μl nuclease-free water and stored at -
434 80°C. The 16S and 18S rRNA genes were PCR amplified using primers 8F/1492R and
435 primers Euk_A/Euk_B primers, respectively. PCR was carried out, the products were cloned
436 into pGEM-T easy (Promega), sequenced and analysed (see 'General *in vivo* and *in vitro*
437 genetic manipulations').

438 **<u>Culture-independent work</u>**

439 **Enrichment to enhance DMSP production in Stiffkey sediment**

440 Microcosm experiments were set up to increase DMSP production and abundance of DMSP-
441 producing organisms from Stiffkey saltmarsh sediment. Microcosms consisted of 2 g of
442 surface sediment slurries in 30 ml MBM media of varying compositions, including a control
443 with MBM (35 PSU, 10 mM NH_4Cl), high salinity (50 PSU), low nitrogen (0.5 mM NH_4Cl),
444 additional MTHB (0.1 mM) or a combination of all three conditions (50 PSU, 0.5 mM
445 NH_4Cl, 0.1 mM MTHB). Samples were incubated at 28ºC for 7 days before quantifying
446 DMSP content (Supplementary Fig. 9). This experiment was scaled up for molecular
447 microbial ecology work using 3 g sediment and 45 ml MBM with either the combined
448 conditions (enriched media; 50 PSU, 0.5 mM NH_4Cl, 0.1 mM MTHB) or control MBM (35
449 PSU, 10 mM NH_4Cl). Sediment slurries were incubated at 28ºC for 14 days. Samples were
450 taken at regular time points, centrifuged and the DMSP content determined in the particulate
451 and cell-free medium (Supplementary Fig. 9). All experiments were done in triplicate.

452 **DNA/RNA extraction and purification**

453 DNA and RNA were extracted from all marine sediment samples (Time 0) and from the 14
454 day incubation sediment (enriched and control samples, see above) following the protocol
455 described by Carrión *et al.*[41]. Samples were stored at -80°C and RNA was purified separately
456 (see below).

457 **Degenerate primer design**

458 To design degenerate primers targeting the *dsyB* gene, 24 DsyB sequences available from
459 Genbank were aligned using the ARB[42] project program to identify conserved amino acid
460 positions. Two non-DsyB sequences with a cut-off value below that used in Curson *et al.*[8,16]
461 were also included in the alignment to guide specific amplification of *dsyB* by the degenerate
462 primers (Supplementary Fig. 17a). Various sets of primers with a degeneracy ≤5 bp spanning
463 different regions of the *dsyB* gene were manually designed. Several different combinations
464 were tested against genomic DNA from positive and negative control strains (Supplementary
465 Table 15). The primer pair dsyB_deg1F and dsyB_deg2R (Supplementary Table 17) yielded
466 a 246 bp fragment from genomic DNA from all positive controls strains tested, with no non-
467 specific bands, and no amplification bands at that size were obtained from any of the negative
468 control strains (Supplementary Fig. 17B). Optimisation of PCR conditions with these primers
469 included annealing temperatures ranging from 60 - 65°C, extension times from 15-60 s and
470 30-40 of cycles. The most specific amplification was obtained with an initial denaturation
471 step of 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, an annealing step of 61°C for
472 15 s and an elongation step of 72°C for 15 s, with a final extension of 72°C for 5 min.

473 Degenerate primers for *mmtN* were designed following the same principles as above with the
474 j-CODEHOP PCR primer design programme[43] using 20 MmtN sequences from Genbank and
475 one MMT sequence (primers were designed to amplify bacterial *mmtN*) (Supplementary Fig.
476 17C). The primers mmtN_degF and mmtN_degR were selected for further analysis

477 (Supplementary Table 17) since they yielded a product of the expected size (301 bp) from
478 four positive control strains, showed the least number of unspecific bands and did not amplify
479 the negative control (Supplementary Fig. 17D). PCR conditions for these primers were
480 optimised using annealing temperatures between 50-60°C, extension times ranging from 20-
481 45 s and 30-35 cycles. The final PCR program consisted of an initial denaturation step of
482 95°C for 3 min, 35 cycles of 95°C for 20 s, annealing at 54°C for 30 s and elongation at 72°C
483 for 30 s, ending in a final extension of 72°C for 7 min.

484 **Quantitative PCR and reverse transcription qPCR (RT-qPCR)**

485 To study the abundance of *dsyB* and *mmtN* transcripts, RNA from environmental samples
486 was purified using the RNase-free kit (Qiagen) and the RNeasy mini kit (Qiagen) according
487 to the manufacturer's instructions. Absence of DNA in RNA samples was confirmed by PCR
488 using primers 27F/1492R[44] (Supplementary Table 17). Purified RNA samples were
489 quantified with a Qubit RNA HS assay kit (Thermo Fisher Scientific). Reverse transcription
490 of RNA was performed with ~100 µg purified RNA. Between 1-9 µl RNA were mixed with 1
491 µl 10 µM specific reverse primer (Supplementary Table 17) as in Farhan Ul Haque *et al* [45].
492 The mixture was incubated for 5 min at 70°C and cooled briefly on ice. Then, 1 µl dNTPs (10
493 mM), 4 µl M-MLV 5 x reaction buffer (Promega), 0.4 µl RNase Inhibitor (40 U/µl, Roche),
494 0.8 µl M-MLV reverse transcriptase (200 U/µl, Promega) and 3.8 µl nuclease-free water were
495 added to the mixture. Finally, samples were incubated at 42°C for 1 h and resultant cDNA
496 was stored at - 20°C until use.

497 qPCR and RT-qPCR assays were performed using a C1000 Thermal cycler equipped with a
498 CFX96 Real-time PCR detection system (BioRad). qPCR reactions (20 µl) contained 2 µl of
499 cDNA/DNA (2-10 ng for 16S rRNA gene and 10-50 ng for *dsyB/mmtN*), 0.8 µl of each
500 primer (10 µM) and 10 µl of SensiFAST™ SYBR® Hi-ROX Kit (Bioline). The primers used

501 in qPCR/RT-qPCR are described in Supplementary Table 17. The qPCR and RT-qPCR
502 reactions consisted of an initial denaturation step of 95°C for 3 min, followed by 40 cycles of
503 95°C for 20 s, 55°C (16S rRNA gene)/60°C (*dsyB*)/54°C (*mmtN*) for 20 s and 72°C for 30s.
504 Specificity of qPCR and RT-qPCR reactions was determined from melting curves from 60-
505 95°C, followed by gel electrophoresis and clone library construction from DNA and/or
506 cDNA isolated from environmental samples. Ratified sequences were between 77-100%
507 identity at the derived amino acid level to ratified DsyB proteins[8,16], and 63-73% to ratified
508 MmtN proteins (Supplementary Table 14), respectively.

509 Quantification of *dsyB* and *mmtN* genes/transcripts was performed using a ten-fold dilution
510 series of DNA/cDNA standards. Standards were prepared by cloning the *dsyB/mmtN* genes
511 amplified from DNA extracted from the environment into the pGEMT-Easy vector
512 (Promega) and using this as template DNA. The detection limit of the qPCR and RT-qPCR
513 assays were 20 copies per 20 μl reaction.

514 For each environmental sample, copy numbers of the *dsyB*, *mmtN* and 16S rRNA
515 genes/transcripts in the technical and biological triplicates were averaged and manually
516 detected outliers were excluded from further analysis. In order to adjust for the differing copy
517 number of 16S rRNA genes within prokaryotes, the copy numbers were normalised by
518 dividing by 3.61, the average copy number in prokaryotes[46]. This was used to estimate a
519 predicted percentage of *dsyB/mmtN*-containing bacteria. Statistical analysis was performed
520 using Student's two-tailed *t*-tests in Microsoft Excel.

521 **Analysis of public marine metagenomes and metatranscriptomes for MmtN**

522 Hidden Markov Model (HMM)-based searches for MmtN homologues in metagenome and
523 metatranscriptome datasets were performed as described in Curson *et al*.[16] using HMMER

524 tools (version 3.1, http://hmmer.janelia.org/)[47]. The MmtN protein sequences used as training
525 sequences to create a HMM profile are listed in Supplementary Table 14. HMM searches
526 were performed on OM-RGC database assemblies with an E value cut-off of $1e^{-30}$, and on
527 selected *Tara* Oceans metatranscriptome databases (Supplementary Table 11) with an E
528 value cut-off of $1e^{-5}$. Each potential MmtN sequence was manually curated using BLASTP
529 analysis against the RefSeq database and discounted as a true MmtN sequence if the top hits
530 were not to a recognised MmtN. The unique hits to MmtN in the metagenomes were
531 normalised to the number of RecA sequences returned, giving an estimated percentage of
532 *mmtN*-containing bacteria compared to *dsyB* and other genes involved in sulfur metabolism.
533 For the metatranscriptomes, unique hits were normalised to gene length against the shortest
534 gene, *dddK*.

535 **Phylogenetic analysis of MmtN protein**

536 MmtN amino acid sequences were aligned in MAFFT[48,49] v7 using default settings, then
537 visually checked. Model selection and phylogeny construction were carried out using IQ-
538 TREE v1.5.3[50], implemented in the W-IQ-TREE web interface[51]. The best supported model
539 was LG+G4, and this model was used to build a phylogeny, with 1,000 ultrafast bootstrap
540 replicates[52] used to assess node support. The tree was rooted using the MMT-like sequence
541 from *A. thaliana*, and was formatted using the ggtree[53] package in R[54].

542 **16S rRNA gene amplicon sequencing**

543 16S rRNA gene amplicon sequencing of Stiffkey saltmarsh sediment samples was performed
544 on at least three biological replicates from each condition by MR DNA (Shallowater, TX,
545 USA), as described in Carrión *et al*.[41]. Sequences were clustered into operational taxonomic
546 units (OTUs) at 97% sequence similarity and taxonomy was assigned by BLASTn using a

547 curated NCBI and RDP database. Taxon-assigned data at the genus level for different
548 samples across multiple runs were converted to count tables and joined in QIIME v1.9[55].
549 Samples were analysed at the genus level according to treatment group (Time 0, Control or
550 Enriched). Data were normalised using total sum normalisation to convert raw counts to
551 relative abundances.

552 The 50 most abundant genera were visualised in a bubble plot using R package ggplot2
553 v2.2.1[56]. Taxa with less than 0.01% mean relative abundance across all samples were
554 removed, yielding a total of 330 genera after exclusion of 491 of the original 821 genera.
555 Kruskal-Wallis rank sum test was used to assess if a significant difference existed at least
556 once across treatment groups, then pairwise comparisons were made between treatment
557 groups using Dunn's test with 'BH' *p*-value correction for multiple pairwise comparisons
558 (Supplementary Table 6). Relative abundances of genera of interest were visualised in box
559 plots using the R packages ggpubr v2.0[57], ggplot2 v2.2.1, and cowplot v0.9.2[58]
560 (Supplementary Fig. 10).

561 Rarefaction curves were created using the R package vegan v2.4-6[59] to assess the sampling
562 depth with average number of species (richness) plotted against number of reads sampled
563 (Supplementary Fig. 3). The number of genera were plotted as a function of an even rarefied
564 sampling depth of 36,066 sequence counts per sample. Data were normalised using total sum
565 normalisation to convert raw counts to relative sequence abundances. Differences between
566 DMSP-producing genera across treatment groups and DMSP gene categories were assessed
567 for normality using the Shapiro-Wilks test, followed by analysis of variance, and Tukey
568 multiple comparison of means test with a 95% confidence interval in the statistical package
569 R[54].

570 ***dsyB* diversity**

571 To the study the diversity of the *dsyB* gene in environmental samples, extracted DNA was
572 subjected to amplification with *dsyB* degenerate primers (dsyB_deg1F and dsyB_deg2R) and
573 subsequently sequenced by MrDNA (Shallowater, Texas, USA) using Illumina MiSeq
574 technology. Sequences were then analysed with QIIME[55] (Macqiime,version 1.9.0) to map
575 the reads to a reference database constructed from 113 ratified DsyB amino acid sequences,
576 with a 55% identity cutoff. Analysis yielded a total of 78,779 quality-filtered sequences with
577 an average of 7,878 reads per sample. The resultant OTU table was sorted using an ID-
578 mapping file identifying the phylogeny for each sequence.

579 **Metagenomic analysis of Stiffkey saltmarsh sediment samples**

580 DNA extracted from three biological replicates of Stiffkey saltmarsh sediment samples at
581 Time 0 and samples incubated for 14 days under control or enriched conditions were
582 combined in equal proportions to perform metagenomic analysis. Library construction and
583 sequencing was conducted by MrDNA (Shallowater, Texas, USA) using Illumina HiSeq
584 technology, as described in Carrión *et al* [41]. Following library preparation, the final
585 concentration of the library was measured using the Qubit® dsDNA HS Assay Kit (Life
586 Technologies), and the average library fragment size was determined using the Agilent 2100
587 Bioanalyzer (Agilent Technologies). For Time 0 samples the average size was 826 bp, 931 bp
588 for Control samples and 1,364 bp for Enriched samples. The library was pooled in equimolar
589 ratios (2 nM), and sequenced paired end for 300 cycles using the HiSeq 2500 system
590 (Illumina). Reads were quality-filtered and trimmed using Trimmomatic[60], obtaining an
591 average of 15,363,915 reads per sample with an average length of 151 bp. Metagenomes
592 were then assembled using SPAdes[61] assembler with kmers 55 to 127 and assemblies were
593 analysed using Quast[62]. N50 values were ~1 kb for all metagenomes assemblies.

594 The abundance of functional genes in unassembled metagenomes was determined by Profile
595 HMM-based searches (see 'Analysis of public marine metagenomes and metatranscriptomes
596 for MmtN') of selected ratified gene sequences (*dsyB, mmtN, DSYB, Alma1, ddd* genes)
597 against the raw reads ($E \leq e^{-4}$). Peptide databases were created by translating merged reads
598 above 20 amino acids in length using the translate function in Sean Eddy's squid package
599 (http://eddylab.org/software.html), as in Curson *et al.*[16]. Only unique hits were counted. The
600 number of unique hits was normalised to read number of the smallest sample and to gene
601 length, and bacterial genes were also normalised to number of RecA hits.

602 **Cultivation studies**

603 **Media and growth conditions for bacteria**

604 *Thalassospira profundimaris* DSM17430*, Pseudobacteriovorax antillogorgiicola*
605 DSM103413, *Roseovarius indicus* DSM26383, *Labrenzia aggregata* LZB033, *Pelagibaca*
606 *bermudensis* HTCC2597, *Novosphingobium* sp. BW1 and the other bacteria isolated from
607 Stiffkey were grown in YTSS[63] or Difco Marine Broth 2216 (BD Life Sciences) complete
608 medium, or MBM[64] (marine basal medium, adjusted to salinity of 35 PSU) (10 mM mixed
609 carbon source from a 1 M stock of 200 mM succinate, glucose, pyruvate, sucrose and
610 glycerol, and 0.5 or 10 mM NH_4Cl as nitrogen source as indicated) at 30°C. *Streptomyces*
611 *mobaraensis* DSM40847 was grown in GYM *Streptomyces* medium (4 g glucose, 4 g yeast
612 extract, 10 g malt extract, 2 g calcium carbonate, 12 g agar per litre distilled water) at 25°C
613 and *Nocardiopsis chromatogenes* DSM44844 was grown in MYM medium (4 g glucose, 4 g
614 yeast extract, 10 g malt extract, 2 g calcium carbonate, 10 g NaCl, 12 g agar per litre distilled
615 water) at 37°C. *Corallococcus coralloides* DSM2259, *Stigmatella aurantiaca* DSM17044
616 and *Myxococcus fulvus* DSM16525 were grown in VY/2 medium (DSMZ medium 9) at
617 30°C. Where indicated, the salinity of MBM was adjusted by altering the amount of sea salts

618 (Sigma-Aldrich) added, and nitrogen levels were altered by adjusting the amount of NH_4Cl
619 added. Methylated sulfur compounds, namely DMSP pathway intermediates, were added to
620 MBM in the *Novosphingobium* intermediate incubation experiment. *Escherichia coli* was
621 grown in Luria-Bertani (LB)[65] complete medium at 37°C. *Rhizobium leguminosarum* was
622 grown in tryptone yeast (TY)[66] complete medium or Y[66] minimal medium (with 10 mM
623 succinate as carbon source and 10 mM NH_4Cl as nitrogen source) at 28°C. Where necessary,
624 antibiotics were added to media at the following concentrations: streptomycin (400 µg ml^{-1}),
625 kanamycin (20 µg ml^{-1}), spectinomycin (200 µg ml^{-1}), gentamicin (20 µg ml^{-1}), ampicillin
626 (100 µg ml^{-1}), rifampicin (400 µg ml^{-1}). Strains used in this study are listed in Supplementary
627 Table 15.

628 **Isolation and characterisation of DMSP-producing bacteria**

629 The 'Time 0' and 'Enriched' samples from Stiffkey sediment enrichment experiments, see
630 above, were serially diluted and plated onto MBM minimal medium. Plates were incubated at
631 28ºC for 72 h. Over 100 single colonies with different morphologies were purified and tested
632 for DMSP production. Isolates of interest were identified by 16S rRNA gene amplification
633 (using 27F/1492R) and sequencing as in Carrión *et al*.[41]. Bacterial isolates or type strains
634 were assayed for DMSP production after 48 h growth in MBM (salinity 35 PSU, 0.5 mM
635 NH_4Cl) by alkaline lysis and GC headspace analysis, see 'Quantification of
636 DMS/DMSP/SMM by GC'. Where indicated, strains were instead either grown in MB
637 medium or cells were scraped from MB agar plates into MBM medium prior to DMSP assays
638 by GC. Cellular protein content was determined using the Bradford method (BioRad). *dsyB*
639 degenerate primers were used to screen isolates for the presence of the gene (see 'Degenerate
640 primer design').

641 **Genome sequencing of Stiffkey isolates**

642 Genomic DNA from *Novosphingobium* sp. BW1, *Stappia* sp. BW2, *Rhodobacterales*
643 bacterium sp. BW5, *Marinobacter* sp. BW6 and *Rhodobacter* sp. BW8 was sequenced by
644 MicrobesNG (Birmingham, UK) using Illumina technology. Resultant reads were trimmed
645 with Trimmomatic[60] and quality-assessed using in-house scripts combined with the following
646 software: Samtools[67], BedTools[68] and bwa-mem[69]. Annotation was performed with RAST,
647 the NMPDR, SEED-based, prokaryotic genome annotation service (http://rast.nmpdr.org)[70],
648 using the genome of the closest related strain as a reference.

649 **DMSP production by cell lysates**

650 For *Novosphingobium* cell lysate experiments, cultures were grown overnight in 5 ml YTSS
651 medium, harvested by centrifugation at 20,000 *g* on a benchtop centrifuge for 5 mins and
652 resuspended in 1 ml 50 mM Tris-HCl buffer (pH 7.5). Samples were sonicated to lyse the
653 cells, then centrifuged at 20,000 *g* for 5 mins to pellet debris, and the lysate was removed.
654 This lysate was dialysed to remove any pre-existing metabolites, using dialysis tubing (3,500
655 Da molecular weight cut-off, SpectrumLabs) in 2 l of dialysis buffer (20 mM HEPES, 150
656 mM NaCl, pH 7.5) at 4°C overnight. From this lysate 2 x 200 µl was mixed with either 1 mM
657 SAM, 1 mM L-Met, or both, and then incubated for 30 min at room temperature. After
658 incubation, assays were immediately transferred to 2 ml gas-tight GC vials, mixed with 100
659 µl 10 M NaOH and MMT activity was measured by GC, alongside heat-killed and buffer
660 only controls (see 'Quantification of DMS/DMSP/SMM by gas chromatography').

661 In order to detect Met, SMM and DMSP-amine in *Novosphingobium* cell extracts, cultures
662 were inoculated to a 5 ml YTSS starter culture and grown for 20 hours at 30°C. The starter
663 culture was then centrifuged at 5,000 *g* for 1 min and resuspended in MBM medium twice.
664 Washed *Novosphingobium* cells were then used to inoculate 1:100 to 100 ml MBM medium
665 (35 PSU, 0.5 mM NH_4Cl, 10 mM mixed carbon source, 0.5 mM Met) and incubated at 30°C

666 for 20 hours. The entire 100 ml culture was then centrifuged at 5,000 *g* for 10 minutes and the

667 cells were resuspended in 1 ml extraction buffer (50 mM potassium phosphate, 5 mM

668 dithiothreitol, 1 mM Na_2EDTA, 0.1 mM pyridoxal phosphate, 5 mM L-ascorbic acid, pH

669 7.2). Cells were sonicated (6 x 15 s) on ice using a Markson GE50 Ultrasonic Processor set to

670 an output of 70. Sonicated *Novosphingobium* cells were centrifuged at 20,000 *g* on a

671 benchtop centrifuge for 5 minutes and the supernatant was retained as cell extracts. Cell

672 extracts were then analysed for Met, SMM and DMSP-amine as described in 'Quantification

673 of Met, DMSP and SMM by HPLC' after 50- and 100-fold dilutions (n=2).

674 **Seawater incubation experiments**

675 Triplicate bacterial strains were grown overnight to stationary phase in MBM (for *P.*

676 *bermudensis* and *Novosphingobium* sp. BW1) or YTSS (*L. aggregata* wild type and *dsyB*$^-$

677 strains, Supplementary Table 15). The cultures were harvested and washed three times with

678 0.2 µm filter-sterilised surface seawater (collected from Yarmouth estuary, latitude 52.6525,

679 longitude 1.7336, September 2016 {0.07 ±0.001 nmol DMSP} for *L. aggregata* work or from

680 Zhanqiao Pier, Qingdao, January 2018 {0.26 ±0.03 nmol DMSP }, for *P. bermudensis* and

681 *Novosphingobium* sp. BW1 work). The resuspended cultures were adjusted to an OD_{600} of 0.4

682 and diluted 1:100 into 20 ml filter-sterilised seawater (T0), followed by incubation at 25°C

683 for 21 h (T1) and 43 h (T2, not done for *L. aggregata*). From the T0, T1 and T2 samples,

684 bacterial cells were harvested by centrifugation at 5,000 *g* for 5 mins and cell-free

685 supernatants collected. The cell pellet was resuspended in 5 ml Tris-HCl buffer (50 mM, pH

686 7.5) and 500 µl 10 M NaOH was added (to chemically lyse the DMSP) to 2 ml of

687 resuspended cells and cell-free supernatants in gas-tight vials and incubated in the dark

688 overnight. Generated DMS was processed by a modified purge and trap method described by

689 Zhang *et al*.[30] and measured by GC, as above. There were no significant changes in DMSP
690 content in seawater only controls.

691 ***In vivo* and *in vitro* genetic manipulations**

692 Plasmids were transferred to *E. coli* by transformation, or to *R. leguminosarum* J391 or *T.*
693 *profundimaris* DSM17430 by conjugation in a triparental mating, using the helper plasmid
694 pRK2013[71]. Restriction enzyme reactions and ligations for cloning were done using Roche
695 enzymes according to the manufacturer's instructions. Standard PCR reactions were
696 performed using 2 x MyFi mastermix (Bioline). PCR products for sequencing or cloning
697 were purified using a Roche High Pure PCR purification kit. The oligonucleotide primers
698 used for molecular cloning were synthesised by Eurofins Genomics and are detailed in
699 Supplementary Table 17. Plasmids and PCR products were sequenced by Eurofins Genomics.
700 The amplified PCR products were then cloned into pLMB509, a vector used for expression in
701 *T. profundimaris*, or pET21a, an IPTG-inducible plasmid for the expression of genes in *E.*
702 *coli*, using *Nde*I and *Bam*HI or *Eco*RI restriction enzymes. All plasmid clones are described
703 in Supplementary Table 16.

704 **Library construction and cosmid screening of *Novosphingobium* sp. BW1**

705 A genomic library of *Novosphingobium* sp. BW1 was constructed essentially as described in
706 Carrión *et al*.[72]. *Novosphingobium* genomic DNA was extracted and partially digested with
707 *Eco*RI, ligated into the wide host-range cosmid vector pLAFR3 and transfected into *E. coli*
708 strain 803, to construct a library with an estimated 90,000 clones. The clones were transferred
709 *en masse* to *R. leguminosarum* J391 by conjugation. A total of 750 transconjugants were
710 picked to MBM medium containing 0.5 mM L-Met and screened by GC (see section
711 'Quantification of DMS/DMSP/SMM by GC') for those containing SMM (as a result of

712 conferred MMT activity). Two clones were identified that conferred MMT activity to *R.*
713 *leguminosarum* J391, clone pBIO2279 and pBIO2280 (Supplementary Tables 8 and 16).

714 **Identification of *mmtN***

715 The inserts in pBIO2279 and pBIO2280 were determined by sequencing their termini and
716 aligning the sequence to the annotated genome sequences of *Novosphingobium* sp. MBES04
717 and BW1. Where the two fragments overlapped, the annotated genes were analysed by
718 BLAST for candidate methyltransferase genes. The *mmtN* gene located was subcloned into
719 pET21a and was shown to confer MMT activity to *E. coli*, as detailed above (Supplementary
720 Table 8).

721 **Identification of MmtN/MMT proteins in databases**

722 BLAST searches[72] to identify homologues of the *Novosphingobium* sp. BW1 MmtN protein
723 were performed using BLASTP at NCBI or JGI, as in Curson *et al*[16]. Representative strains
724 containing MmtN homologues (E values $\leq 1e^{-50}$) were obtained, shown to produce DMSP
725 and/or their *mmtN* genes were cloned and shown to confer MMT activity (as above). Thus, an
726 E value of $\leq 1e^{-5}$ to a functional MmtN protein (Supplementary Table 14) was used as the cut-
727 off to predict MMT functionality. Bacterial sequences with significant similarity to the larger
728 plant-like MMT enzymes were identified using BLASTP, using the *Zea mays* MMT protein
729 (NCBI accession: NP_001104941) as the query. Representative strains containing these
730 MMT-like enzymes were obtained and assayed for DMSP production, as above.

731 **Methionine *S*-methyltransferase (MMT) assays**

732 To measure MMT activity from pET21a clones expressing the *mmtN* gene in *E. coli* BL21
733 (Supplementary Table 16), cultures were grown (in triplicate) overnight in LB medium, and

734 diluted 1:100 into 5 ml LB and incubated for 2 h at 37°C. This was then induced with a final
735 concentration of 0.2 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) (Sigma-Aldrich) and
736 incubated at 30°C overnight. For each culture, L-Met (Sigma-Aldrich) was added to 1 ml of
737 culture (final concentration 0.5 mM) and incubated for 8 h at 30°C before assaying for SMM
738 production by GC (see 'Quantification of DMS/DMSP/SMM by gas chromatography').
739 Protein concentrations were determined using the Bradford method (BioRad). *E. coli* BL21
740 containing the empty pET21a vector was used as a control.

741 **MmtN protein purification and enzyme characterisation**

742 Cultures of *E. coli* BL21 containing pBIO21N1 were grown in LB medium at 37°C, to an
743 OD_{600} of 0.6–0.8, then induced at 20°C for 16 h with 0.3 mM IPTG. The MmtN protein was
744 purified with Ni^{2+}-NTA resin (QIAGEN, Germany), and then fractionated using gel filtration
745 buffer (10 mM Tris-HCl and 100 mM NaCl, [pH 8.0]) on a Superdex-200 column (GE
746 Healthcare). MmtN purification was carried out at 4°C. For the Ni^{2+}-NTA resin purification,
747 wash buffer (50 mM Tris-HCl, 250 mM NaCl and 20 mM imidazole, [pH 8.0]) was used to
748 remove protein impurities, followed by the elution buffer (50 mM Tris-HCl, 250 mM NaCl
749 and 250 mM imidazole, [pH 8.0]) to elute the purified protein from the column. MmtN
750 enzyme activity was measured by monitoring the production of SAH (S-adenosyl
751 homocysteine) produced by the demethylation of SAM, detected by HPLC through its UV
752 absorbance under 260 nm. The standards for SAM and SAH were purchased from New
753 England Biolabs, and Sigma-Aldrich respectively. During the reaction, the SAM was added
754 in excess, and a standard curve of SAH was generated from a 1 mM stock that was diluted to
755 concentrations in a range of 0-50 μM. Based on the standard curve, the peak area of SAH on
756 HPLC was converted to SAH concentrations. Several different detection conditions were
757 trialled, varying UV lengths and different phases to establish the following method for SAH

758 detection: SAH was measured by HPLC (Ultimate 3000, Dionex, America) on a SunFire C18
759 column (Waters, America) with a linear gradient of 1–20% acetonitrile in 50 mM ammonium
760 acetate (pH 5.5) over 24 min at 260 nm.

761 LC-MS was used to confirm that SMM is produced when the pure MmtN enzyme *S*-
762 methylates Met, using SAM as the methyl donor (Supplementary Fig. 13a). Optimal MmtN
763 activity was determined by testing temperature and pH conditions, and comparing enzyme
764 activity, with the highest activity defining 100% activity, and other tested conditions
765 described as relative to it. The reaction mixtures were incubated at temperature intervals of
766 10°C, from 0°C to 60°C, for 30 min. For optimal pH levels, MmtN activity was examined
767 using Britton–Robinson buffer (40 mM H_3BO_3, 40 mM H_3PO_4 and 40 mM CH_3COOH), at
768 pH values between pH 5.0 and pH 10.0. Optimum conditions were pH 8.0, 30°C. In each of
769 these assays, MmtN protein was used at the concentration indicated. Kinetic parameters (K_M)
770 were determined by non-linear analysis, based on the initial rates and determined using 3.34
771 µM MmtN and 0.1-4 mM SAM, or 0.1-6 mM L-Met. The reaction mixture was incubated at
772 30°C for 30 min before detection. The enzyme activities were linear with respect to
773 incubation time and enzyme concentration. Origin 8.5 was used to calculate K_M.

774 **Gene mutagenesis in *T. profundimaris* DSM17430**

775 *Novosphingobium* sp. BW1 was resistant to many antibiotics so *T. profundimaris* DSM17430
776 was used for gene knock-out experiments. Primers were designed (Supplementary Table 17)
777 to amplify fragments internal to the *T. profundimaris* DSM17430 *mmtN* gene
778 (WP_008888945, TH2_03115) and a closely linked aminotransferase (TH2_03140), which
779 were cloned into pBIO1879[74], a derivative of the suicide vector pK19mob[75]. The resulting
780 clones (Supplementary Table 16) were transferred into a spontaneous rifamipicin-resistant
781 derivative (strain J595) of *T. profundimaris* DSM17430 by tri-parental conjugation using the

782 helper strain *E. coli* pRK2013. The *T. profundimaris* gene insertional mutants J596 (*mmtN*⁻
783 mutant) and J597 (aminotransferase mutant) were isolated on YTSS agar containing
784 rifampicin (J595), kanamycin (pBIO1879) and spectinomycin (pBIO1879). All mutants were
785 ratified by PCR and checked for their ability to synthesise DMSP.

786 To confirm that the *mmtN* mutation in *mmtN*⁻ mutant strain J596 (Supplementary Table 14)
787 was responsible for the loss of DMSP production phenotype, cloned *Novosphingobium mmtN*
788 (pBIO509N) was mobilised into J596 through tri-parental crossing.

789 **Phenotyping of *T. profundimaris* mutant**

790 Where MBM was used as the minimal medium for the following experiments, this medium
791 lacked any methylated sulfur DMSP pathway intermediates. To identify potential phenotypes
792 for the mutations in *mmtN*, the J595 (wild type) and J596 (*mmtN*⁻) strains were grown with
793 varying levels of salt and nitrogen, or under different environmental conditions, as in Curson
794 *et al.*[8]. Strains were tested against 35 and 50 PSU for salt tolerance and 10, 0.5 or 0.1 mM
795 NH_4Cl for different nitrogen levels, and growth was measured by OD_{600}. Tolerance to
796 freezing was also tested, as in Curson *et al.*[8]. Competition experiments were performed in
797 which cultures of the wild type and mutant strains were grown to stationary phase in 35 PSU
798 MBM (10 mM NH_4Cl), OD_{600} adjusted, mixed in equal parts (500 µl of both) and subjected
799 to high salinity (50 PSU) and reduced nitrogen (0.5 mM). Prior to and after perturbation,
800 aliquots of the mix were serially diluted and plated on MB agar. Single colonies were tested
801 for kanamycin/spectinomycin resistance (mutant selection) to distinguish the wild type from
802 the mutant strain. All the above experiments used three biological replicates for each
803 condition.

804 **Data Availability**

805 The 16S rRNA gene amplicon sequencing, metagenomic data and whole genome sequences
806 generated in this study are publicly available at NCBI single read archive (BioProject
807 PRJNA522699).

808

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999

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1012

1013 **Author contributions**

1014 J.D.T. wrote the paper, designed all experiments and did experiments; B.T.W. wrote the

1015 paper, designed all experiments and did/contributed to all experiments and prepared

1016 figures/tables; K.C. did experiments (genomic library screening, mutant complementation

1017 and characterised MMT^+ bacteria); A.B.M. did experiments (LC-MS work); A.R.J.C. did

1018 experiments (genomic library construction, MMT assays, mutant construction, rate
1019 experiments); Y.Z. did experiments (qPCR, degenerate primer design, sampling and DMSP
1020 quantification in Mariana Trench); J.L. and J.L. did experiments (seawater incubations,
1021 qPCR, sediment sampling, purge-trap analysis, DNA/RNA purification from water); S.N-P.,
1022 M.P. and C.Y.L. designed and did experiments (MmtN protein characterisation); P.P.L.R. did
1023 experiments (DMSP quantification in sediment, isolation and characterisation of eukaryotic
1024 species); L.G.S. wrote the paper and did experiments (evolutionary analysis of MmtN
1025 sequences and phylogenetic tree construction); C.A.B. devised experiments for measuring
1026 DMSP pathway intermediates in sediment and cell lysate by HPLC, carried out LC-MS
1027 experiments and discussed results; B.W.M. did experiments (16S rRNA amplicon sequencing
1028 analysis) and prepared figures; B.P. did experiments (cell lysate assays); J.P. did experiments
1029 (degenerate primer design, sediment sampling, bioinformatics analysis of metagenomic
1030 sequencing); O.C., X-H.Z., Y-Z. Z, J.C.M. designed experiments and discussed results.

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1032 **Additional information**

1033 Supplementary information is available for paper. Reprints and permissions information is
1034 available at www.nature.com/reprints.

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1036 **Competing interests**

1037 The authors declare no competing financial interests.

1038 **Figure 1. DMSP synthesis in tested marine sediments.** (a and b) The mean standing stock
1039 concentration of DMSP in surface sediment (brown) and the overlying water (blue) from two
1040 saltmarshes Stiffkey and Cley, from an estuary (Yarmouth), and from the surface seawater
1041 (blue) and 4,500 m deep surface sediment (red) from the Challenger Deep of the Mariana
1042 trench (n=3 biologically independent samples). (c) qPCR work done on DNA (qPCR) and on
1043 mRNA (RT-qPCR) isolated from Stiffkey saltmarsh natural sediment (T0) and incubated
1044 sediment samples (control {CON}and enriched {ENR}for DMSP production); Yarmouth
1045 estuary sediment, Cley saltmarsh sediment and Mariana Trench 4,500 m deep surface
1046 sediment samples; and on Stiffkey saltmarsh pond water and coastal Great Yarmouth
1047 seawater samples. qPCR was done using degenerate primers designed to the DMSP synthesis
1048 genes *dsyB* and *mmtN* (n=2 and n=3 independent samples, the black line represents the mean
1049 value).

1050 **Table 1. DMSP synthesis rates and DMS production after 24 h using ^{3}H-Methionine,**

1051 **determined from saltmarsh and estuary samples from North Norfolk.**

Sampling site	DMSP synthesis rate (fmol DMSP g^{-1} min^{-1} or ml^{-1} min^{-1})	pmol DMSP m^{-2} h^{-1}	pmol DMS g^{-1} captured after 24h
Stiffkey			
Sediment	263	158	1.89
Water	0.57		
Yarmouth			
Sediment	135	81.5	0.04
Water	2.27		
Cley			
Sediment	145	85.8	1.89
Water	1.13		

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1053 **Figure 2. DMSP biosynthesis pathways and bacterial DMSP production.** (a) Predicted
1054 pathways for DMSP biosynthesis edited from Curson *et al*[8] in higher plants and bacteria
1055 containing *mmtN* (*Spartina,**SMM is converted to DMSP-aldehyde (DMSP-ald) via an
1056 unconfirmed process in *Wollastonia*) (left); macroalgae (*Ulva*, *Enteromorpha*), diatoms
1057 (*Thalassiosira*, *Melosira*), prymnesiophytes (*Emiliania*), prasinophytes (*Tetraselmis*)
1058 (centre), algae that contain *DSYB* and bacteria that contain *dsyB*; and the dinoflagellate
1059 *Crypthecodinium* (right). The dotted line represents a suggested but as yet unconfirmed
1060 pathway. Enzymes involved in the *Spartina* pathway are in blue (MMT, methionine
1061 methyltransferase; SDC, *S*-methylmethionine decarboxylase; DOX, DMSP-amine oxidase;
1062 DDH, DMSP-aldehyde dehydrogenase). Abbreviations: SMM, *S*-methylmethionine, Met,
1063 methionine; MTOB, 4-methylthio-2-oxobutyrate; MTHB, 4-methylthio-2-hydroxybutyrate;
1064 DMSHB, 4-dimethylsulfonio-2-hydroxybutyrate, MTPA, 3-methylthiopropylamine, MMPA,
1065 methylmercaptopropionate. (b) *Novosphingobium* sp. BW1 DMSP production with or
1066 without (control) pathway intermediates (0.5 mM) in MBM minimal medium (10 mM
1067 succinate as carbon source, 10 mM NH_4Cl as nitrogen source). (n=4 independent samples,
1068 the black line represents the mean value). Student's two-tailed *t*-test ($P<0.05$): Met ($p=0.001$),
1069 SMM ($p=0.000001$) and MTOB ($p=0.0002$) were all significantly different to no addition
1070 (Control). (c) LC-MS chromatograms for DMSP (m/z 135) and GBT (glycine betaine) (m/z
1071 118) in *Thalassospira profundimaris* J595 wild type (contains *mmtN*), compared to the J596
1072 *mmtN*$^-$ mutant. These experiments were repeated twice with similar results.

1073 **Figure 3. Maximum-likelihood phylogenetic tree of MmtN proteins.** Species are colour-
1074 coded according to taxonomic class as shown in the key, with proteins shown to be functional
1075 marked with an asterisk. Bootstrap support for nodes is marked. Bacterial MmtN proteins are
1076 boxed in blue and the larger MMT proteins are boxed in cream. Based on 47 protein
1077 sequences.

a

Cley

Stiffkey

1.1 nmol ml^{-1}
91 nmol g^{-1}

0.66 nmol ml^{-1}
128 nmol g^{-1}

Norwich

Great Yarmouth

0.91 nmol ml^{-1}
103 nmol g^{-1}

Ipswich

20 km

b

6.3 x 10^{-3} nmol ml^{-1}

5.0 nmol g^{-1}

Mariana Trench

0 m (Depth)
2,000 m
4,000 m
6,000 m
8,000 m
10,000 m

c

dsyB qPCR

dsyB RT-qPCR

mmtN qPCR

Gene abundance (copies g^{-1} or ml^{-1})

1E+9
1E+8
1E+7
1E+6
1E+5
1E+4
1E+3
1E+2
1E+1

Stiffkey (T0) (n=3)
Stiffkey (ENR) (n=3)
Stiffkey (CON) (n=3)
Yarmouth (n=3)
Cley (n=3)
Mariana Trench (n=3)
Pond water (n=3)
Seawater (n=3)

a

Met

METHYLATION
(MMT)
AdoMet
AdoHcy

TRANSAMINATION

DECARBOXYLATION

MTOB

SMM

REDUCTION
NADPH
NADP

MTPA

DECARBOXYLATION
(SDC)

MTHB

TRANSAMINATION - DECARBOXYLATION

DMSP-amine*

Steps to be determined

OXIDATION
(DOX)

METHYLATION
AdoMet
AdoHcy

DMSP-ald

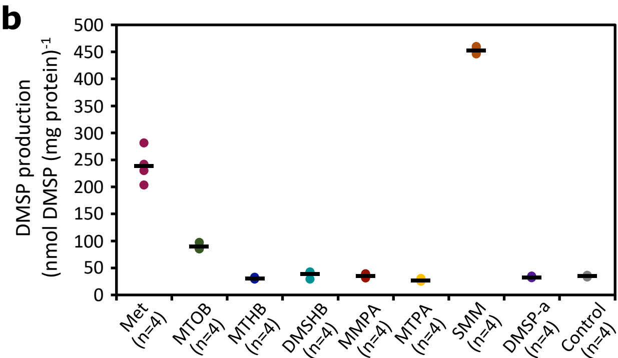
DMSHB

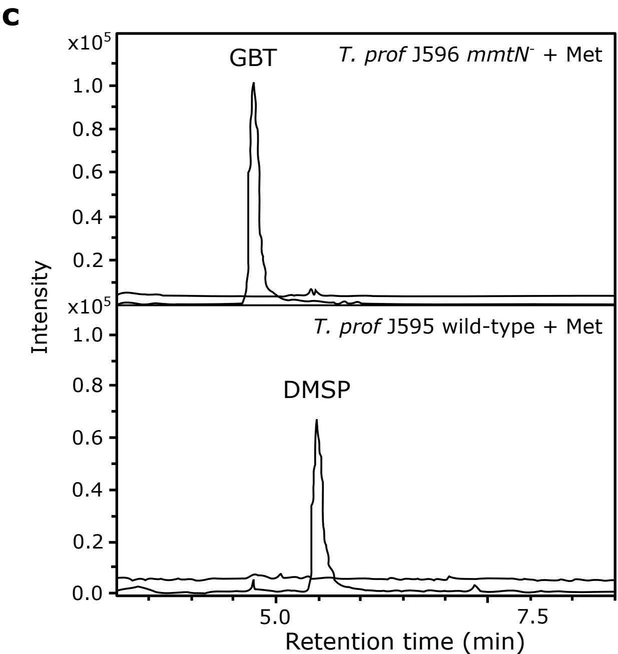
MMPA

DEHYDROGENATION
(DDH)

DECARBOXYLATION

DMSP





Taxon

- Actinobacteria
- Alphaproteobacteria
- Deltaproteobacteria
- Gammaproteobacteria
- Oligoflexia
- Plantae
- Unclassified bacteria

Thalassospira profundimaris WP0211
Thalassospira tepidiphila A03514
MAG assembly *Thalassospira* sp. 2
MAG assembly *Thalassospira* sp. 1
Thalassospira sp. MCCC 1A02898
Thalassospira sp.SAT1308
MAG assembly *Thalassospira* sp. 3
Thalassospira indicus PB8B
Thalassospira sp.DSM26710
Thalassospira sp.SAT187
Thalassospira sp.MCCC1A01148
MAG assembly *Thalassospira* sp. 4
Thalassospira sp.MIT1351
Thalassospira sp.SP131
Thalassospira sp.HJ
Thalassospira australica NP3b2
Thalassospira sp.GB04J01
Thalassospira lucentensis 1A00383
MAG assembly *Novosphingobium* sp. 1
Novosphingobium sp.MBES04
Croceicoccus mobilis Ery22
Novosphingobium sp.CPC302
MAG assembly marine alphaproteobacterium 3
Ponticaulis sp.NAT89
*Ponticaulis sp.*IN28
Labrenzia sp.OB1
Labrenzia marina DSM17023
Labrenzia sp.011
MAG assembly marine alphaproteobacterium 2
MAG assembly marine alphaproteobacterium 1
Alteromonadaceae bacterium 2052S
MAG assembly marine gammaproteobacterium
Nocardiopsis chromatogenes YIM90109
Streptomyces mobaraensis NBRC13819
Saccharothrix syringae NRRLB−16468
Micromonospora nigra 43818
Hartmannibacter diazotrophicus E18T
Agrobacterium vitis S4
Rhodobacter litoris JA431
Rhodobacter aestuarii DSM19945
Sagittula sp.P11
Roseovarius indicus B018
MAG assembly *Roseovarius* sp.
Roseovarius confluentis SAG6
Defluviimonas sp.MS2−2
Candidatus Taylorbacteria CG11