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Supplementary material

Revising upper-ocean sulfur dynamics near Bermuda: new lessons from 3 years of concentration and rate measurements

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DMS yield measurements

Bacterial production of DMS was determined as the conversion of ^{35}S-DMSP to ^{35}S-DMS following the protocols of Kiene and Linn[1] and Slezak *et al*.[2] Briefly, glass serum vials were filled (~14 mL) with whole seawater. Dimethyl disulfide (DMDS) was added to a final concentration of ~300 nM to inhibition the consumption of DMS by heterotrophic bacteria[3] and ^{35}S-DMSP was added for a final concentration of 1000 DPM mL^{-1}. Samples were incubated at in situ surface temperatures in the dark for 24 h. After the incubation period, three subsamples were taken: (1) a 1-mL subsample for total ^{35}S activity (A_{total}), (2) a 5-mL subsample for volatile ^{35}S-DMS (A_{DMS}), and (3) a 5-mL subsample for residual ^{35}S-DMSPd (A_{DMSPd}). Subsample 1 was added directly to EcoLume and counted after a 24-h incubation. The subsample for ^{35}S-DMS analysis (subsample 2) was added to a 35-mL trapping bottle containing 0.1 mL of SDS (10 %) and 50 μL of Elmann's reagent. The ^{35}S-DMS present in the subsample was trapped onto an AE glass fibre filter soaked with 200 μL of 3 % H_2O_2. The ^{35}S-DMSPd subsample (subsample 3) was filtered through a 0.2-μm filter using gentle vacuum and 3.5 mL of the filtrate was added to a plastic acidification vial containing 50 μL of 50 % H_2SO_4. After > 24-h incubation, 200 μL of 5 N NaOH was added to 3 mL of the acidified sample and the resulting ^{35}S-DMS was trapped onto an AE glass fibre filter soaked with 200 μL of 3 % H_2O_2. To ensure complete trapping of ^{35}S-DMS, subsamples 2 and 3 were incubated on a rotary shaker (100 rpm) for > 6 h. The filters were then added to EcoLume and counted after a 24-h incubation. An abiotic control was run using 0.2-μm gravity filtered water. Percentage DMS yield from DMSPd consumption was calculated as:

$$DMS\,yield = \frac{A_{DMS}}{A_{total} - A_{DMSPd}}$$

where A_{total}, A_{DMSPd} and A_{DMS} are defined above and were normalised to a 1-mL subsample. DMSyield$_{abiotic}$ was calculated in the same way using A_{total}, A_{DMSPd} and A_{DMS} from the DMDS control. DMSyield$_{abiotic}$ was then subtracted from the DMSyield value.

In order to accurately quantify gross DMS production from DMSPd consumption, the inhibition of DMS consumption is required. DMDS has been shown to be effective at inhibiting DMS consumption[3]; however, our preliminary results indicated that DMDS also inhibits the production of DMS from DMSPd by heterotrophic bacteria. Specifically, DMDS was added to DMSP lyase potential enzyme assay (see Levine *et al*.[4] for a full description of the potential enzyme assay) and DMS production was reduced by nearly 100 % to essentially zero. Similarly, the heterotrophic DMS production rates determined by the ^{35}S-DMSP method were often less than the abiotic (0.2-μm gravity filtered) controls (Fig. S1). Due to concerns of experimental error, we are not presenting the results from these analyses.

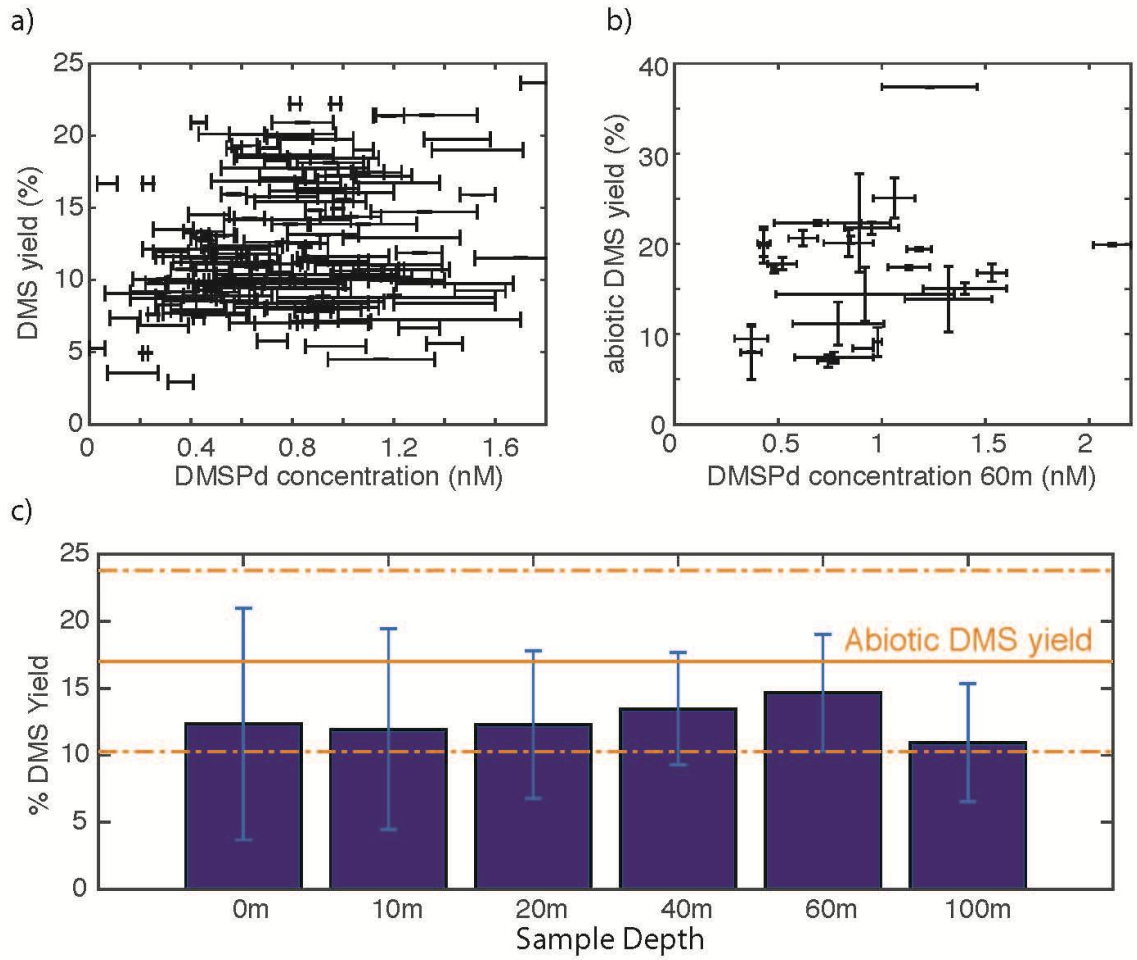

Fig. S1. The relationship between dimethylsulfide (DMS) yield from heterotrophic dimethylsulfoniopropionate (DMSP) consumption *v.* dissolved dimethylsulfoniopropionate (DMSPd) concentration (a) and abiotic dark DMS production and DMSPd concentrations (b). No relationship was observed between DMS yield and DMSPd concentrations and a weak positive relationship was observed between DMSPd concentrations and abiotic DMS yield. The percentage DMS yield was often lower than the abiotic control (c) and showed very little pattern with depth (c) or season (not shown).

DMS biological consumption abiotic control

Bacterial DMS consumption rate constant (k_{DMS}) was calculated as:

$$k_{DMS} = k - k_{ctr}$$

where k is the total loss rate constant and k_{ctr} is the dark abiotic loss rate constant. While some fraction of k_{ctr} may be attributed to incomplete inhibition of DMS consumption by DMDS, assuming 90 % inhibition by DMDS,[3] this contribution can be estimated to be 0.09 ± 0.04 day $^{-1}$ over the entire time-series or about half the observed k_{ctr}. In addition, k_{ctr} showed no correlation with $k_{60\ m}$ (R^2 = 0.008, P = 0.65) suggesting that it is unlikely to be purely a measure of incomplete inhibition. Fig. S2 shows the seasonal variations in k.

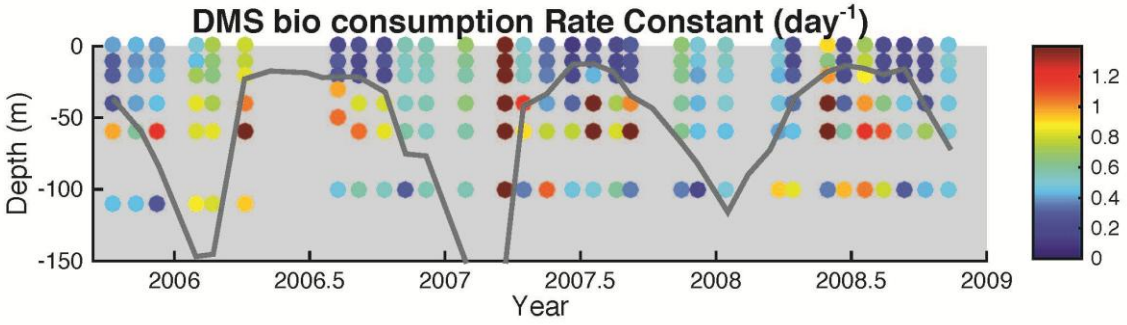

Fig. S2. Total dimethylsulfide (DMS) biological consumption rate constant (k) (day^{-1}) at the Bermuda Atlantic Time-series Study (BATS) site from 2005–2008. This figure displays the observed values of k without subtraction of the abiotic control (k_{ctr}). The mixed layer depth is shown as a grey line.

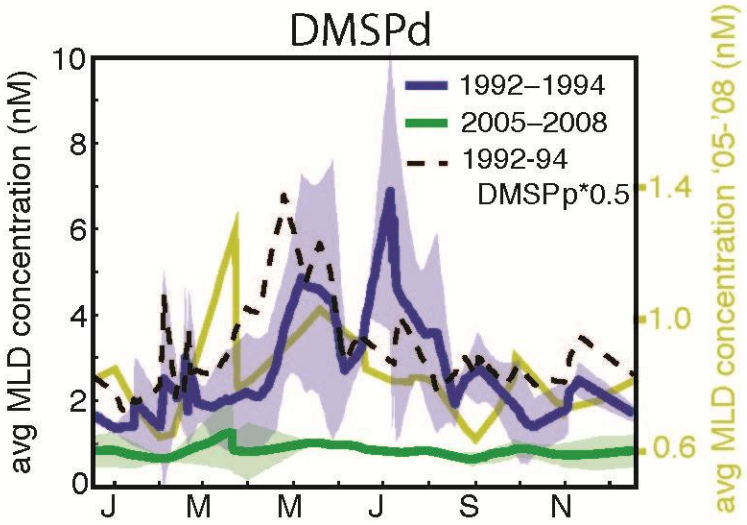

Fig. S3. Comparison between 1992–1994[5] and 2005–2008 (this study) mixed layer dissolved dimethylsulfoniopropionate (DMSPd) (b). The shaded regions denote the 1σ error bars. 2005–2008 DMSPd concentrations are displayed on both the left *y*-axis on the same scale as the 1992–1994 measurements for direct comparison and on the right *y*-axis on a reduced scale to highlight the seasonality in 2005–2008 DMSPd. For reference, 1992–1994 particulate dimethylsulfoniopropionate (DMSPp) concentrations (scaled by 0.5) are also shown. The large difference in DMSPd concentrations between the two datasets is attributed primarily to methodological differences.

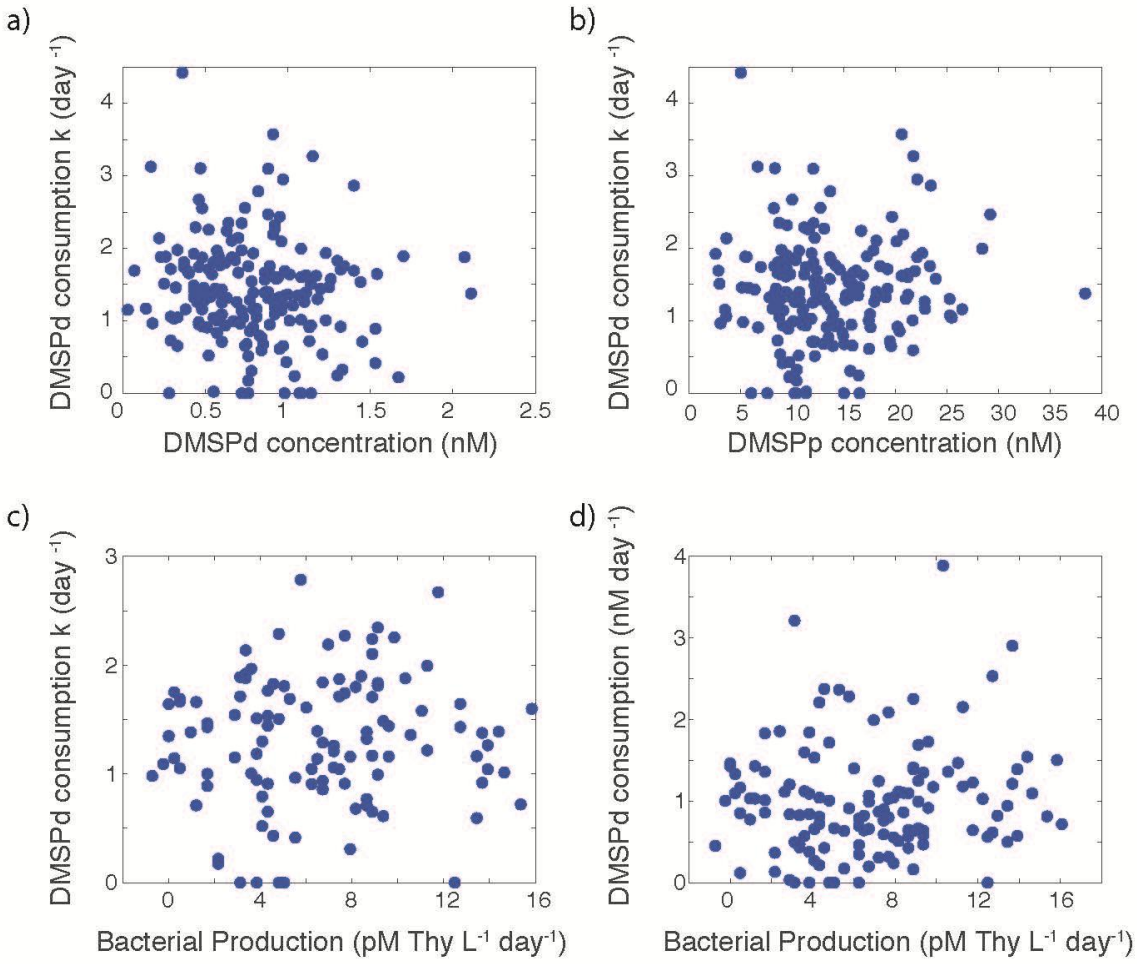

Fig. S4. The lack of correlation between observed dissolved dimethylsulfoniopropionate (DMSPd) consumption rate constant k (day^{-1}) and DMSPd concentration (a), particulate dimethylsulfoniopropionate (DMSPp) concentration (b) and bacterial production as measured by thymidine incorporation (c). Also shown is DMSPd consumption rate ($nM\ day^{-1}$) *v.* bacterial production (d).

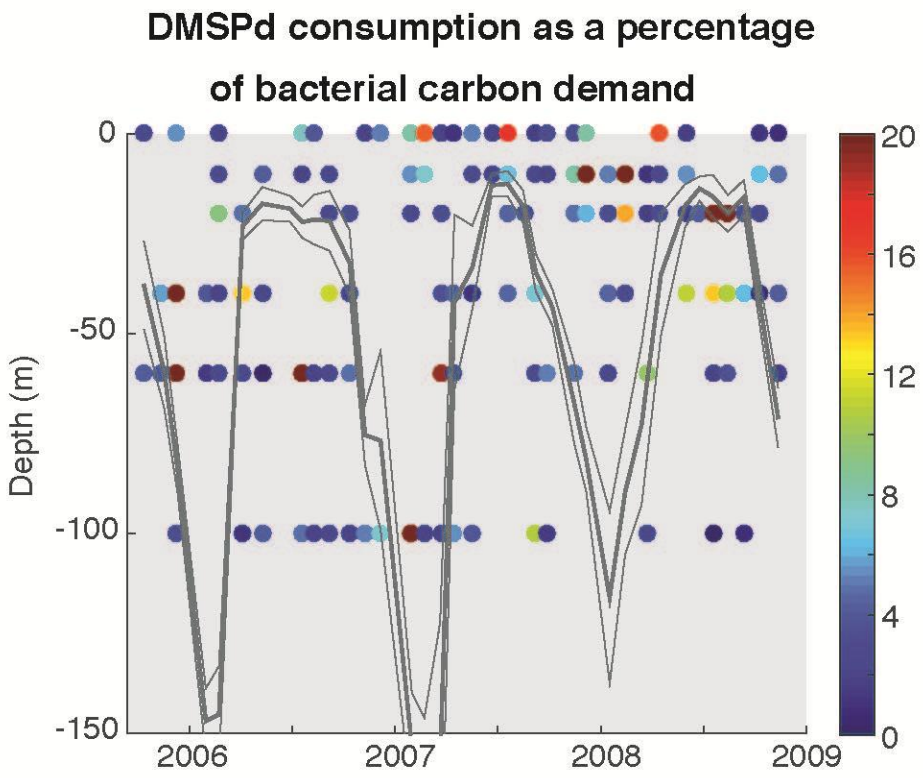

Fig. S5. Percentage of bacterial carbon demand that can be accounted for by dissolved dimethylsulfoniopropionate (DMSPd) consumption at Bermuda Atlantic Time-series Study (BATS) site from 2005 to 2008. Results were calculated using a bacterial growth efficiency of 0.14. The mixed layer depth is shown as a grey line.

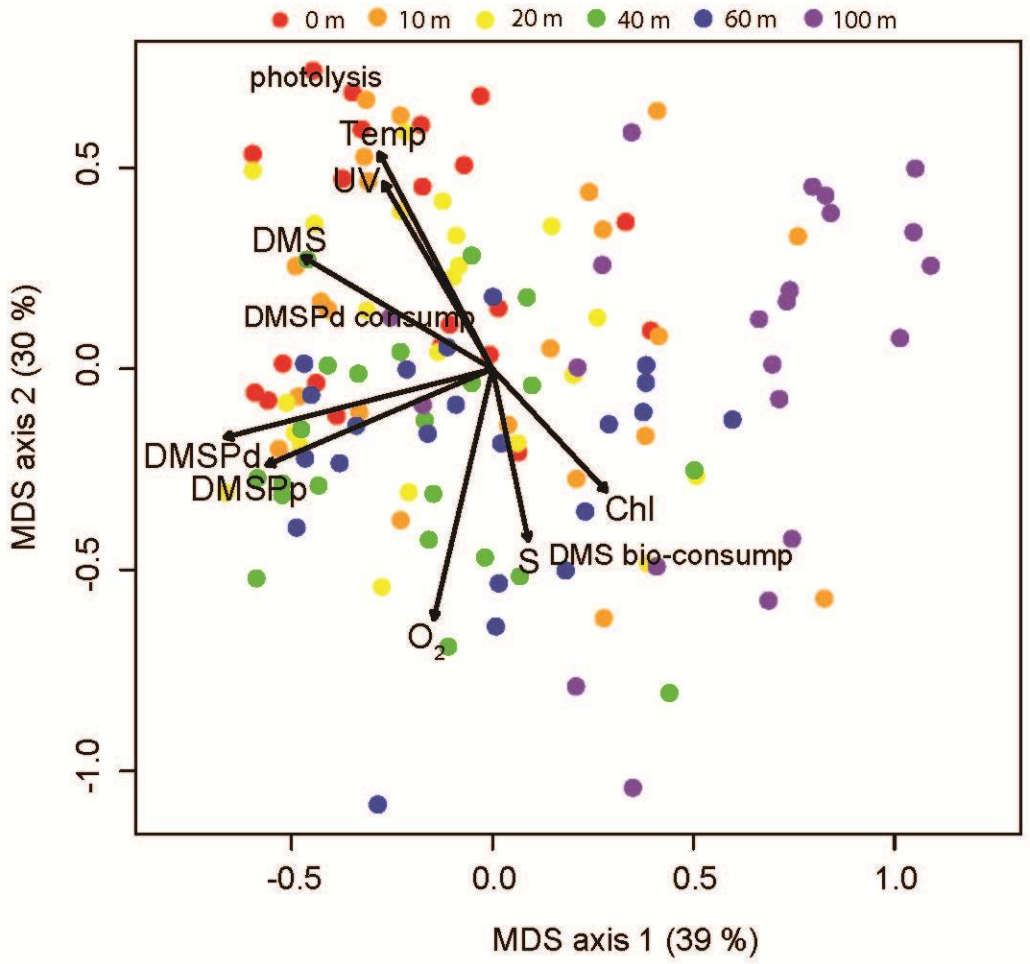

Fig. S6. Multidimensional scaling plot of upper ocean sulfur cycling dynamics. The first and second axis scores for all samples (n = 131) are denoted with the round symbols. The locations of the variable scores for the input variables (particulate dimethylsulfoniopropionate (DMSPp), dissolved dimethylsulfoniopropionate (DMSPd) and dimethylsulfide (DMS) concentrations and DMSPd consumption, DMS biological and photolysis rates) are also given. Environmental variables, temperature (T), salinity (S), oxygen (O_2) and UV dose (UV), are projected onto the axes and shown as black arrows. The samples are colour coded by depth. Axis 1 accounts for 39 % of the total sample variance and axis 2 accounts for 30 % of sample variance. Unlike Fig. 6 (main text), no clear pattern is observed between the environmental parameters and DMS(P) standing stocks or rates. This suggests that variations in DMSPd, DMS and photolysis rate constants provide valuable insight into upper ocean sulphur cycling, which cannot be gained from looking at rates alone.

References

[1] R. P. Kiene, L. J. Linn, The fate of dissolved dimethylsulfoniopropionate (DMSP) in seawater: tracer studies using ^{35}S-DMSP. *Geochim. Cosmochim. Acta* **2000**, *64*, 2797–810. doi:10.1016/S0016-7037(00)00399-9

[2] D. Slezak, R. P. Kiene, D. A. Toole, R. Simó, D. J. Kieber, Effects of solar radiation on the fate of dissolved DMSP and conversion to DMS in seawater. *Aquat. Sci.* **2007**, *69*, 377–93. doi:10.1007/s00027-007-0896-z

[3] G. V. Wolfe, R. P. Kiene, Effects of methylated, organic, and inorganic substrates on microbial consumption of dimethyl sulfide in estuarine waters. *Appl. Environ. Microbiol.* **1993**, *59*, 2723–6.

[4] N. M. Levine, V. A. Varaljay, D. A. Toole, J. W. H. Dacey, S. C. Doney, M. A. Moran, Environmental, biochemical and genetic drivers of DMSP degradation and DMS production in the Sargasso Sea. *Environ. Microbiol.* **2012**, *14*, 1210–23. doi:10.1111/j.1462-2920.2012.02700.x

[5] J. W. H. Dacey, F. A. Howse, A. F. Michaels, S. G. Wakeham, Temporal variability of dimethylsulfide and dimethylsulfoniopropionate in the Sargasso Sea. *Deep Sea Res. Part I Oceanogr. Res. Pap.* **1998**, *45*, 2085. doi:10.1016/S0967-0637(98)00048-X