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Research Paper

Influence of vitamin B_{12} availability on oceanic dimethylsulfide and dimethylsulfoniopropionate

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Environmental context. Cobalamin, or vitamin B_{12}, is receiving increased attention as a critical trace nutrient in the growth and metabolic processes of oceanic phytoplankton and bacterial communities. We present evidence that indicates B_{12} has a more significant role in the biogeochemical cycling of the climatically important compounds dimethylsulfide and dimethylsulfoniopropionate than previously understood. Several possible mechanisms are examined that link cellular-level processes involving B_{12} to global-scale biogeochemical processes involving the oceanic cycling of dimethylsulfoniopropionate and dimethylsulfide.

Abstract. Evidence is presented showing that dissolved dimethylsulfoniopropionate ($DMSP_d$) and dimethylsulfide (DMS) concentrations are influenced by the availability of vitamin B_{12} in two oceanographically distinct regions with different DMS production capacities, the central equatorial Pacific Ocean and the Ross Sea, Antarctica. In both locations, addition of B_{12} to incubation experiments resulted in decreases in DMS and, in some cases, $DMSP_d$ concentrations relative to unamended controls. In no case did increasing iron availability significantly ($\alpha = 0.1$) alter DMS concentrations relative to controls. The relative decreases in DMS between B_{12} addition and control experiments were significant ($\alpha = 0.1$) in five of seven experiments conducted at ambient iron levels. Overall, DMS concentrations were on average 33.4 % (±15.1 %; 1 standard deviation) lower, relative to unamended controls, by the end of incubation experiments when B_{12} was added. Declines in $DMSP_d$ were observed in three of five experiments. Similar trends were observed when B_{12} was added to iron-supplemented bottle incubation experiments (30.4 ± 10.4 % lower final DMS concentrations in $+B_{12}$Fe treatments relative to +Fe treatments). Several possible molecular-level explanations exist for this link between B_{12} and DMS production, including potential B_{12} dependence of methyltransferase enzymes involved in both DMS and DMSP degradation. Although the enzymology of these reactions remains unclear, the relationships described here provide evidence for plausible mechanisms behind the microbial modulation of oceanic DMS.

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Introduction

Oceanic dimethylsulfide (DMS) production and release into the atmosphere is thought to be a significant driver of cloud formation and possible climate feedback mechanisms.[1] Whereas recent arguments have been made against a phytoplankton–DMS–albedo feedback,[2] other studies have concluded that DMS-derived aerosols represent a major fraction of cloud condensation nuclei in marine locations and have the capacity to cause drastic changes in climate.[3] Thus, identifying controls on oceanic DMS release remains critical for understanding the ocean's role in climate regulation. DMS arises from microbial processing of the algal metabolite dimethylsulfoniopropionate (DMSP) and, once released to the dissolved phase ($DMSP_d$), can be cleaved to DMS or demethylated to other sulfur-containing products.[4] Because these compounds contain reduced-sulfur and methyl groups, they also represent an important pathway in the biogeochemical cycling of organic sulfur and the transfer of sulfur through the microbial food web in the marine environment.[4]

The relative proportions of the cleavage and demethylation processes are thought to be an important control on DMS production, but the factors influencing these proportions remain ambiguous.[5,6] Indeed, new enzymes and pathways for DMSP processing are being rapidly described, but controls on their relative rates of activity remain poorly understood. The discovery of a new DMSP-cleaving enzyme in the haptophyte *Emiliania huxleyi*,[7] which is clearly encoded in the algal genome, corroborates earlier measurements of algal DMSP-lyase activity.[8–10] This finding emphasises the idea that the eukaryotic formation of DMS, along with the distinctly different bacterial DMSP cleavage pathways, are important contributors to marine DMS production.[7,11–13]

DMS sinks include loss to the atmosphere, photochemical oxidation and further microbial metabolism.[14–16] Sea-surface DMS concentrations range from sub-nanomolar to hundreds of nanomolar[17] and are generally higher in locations where any of the following conditions occur: high irradiance, high phytoplankton productivity, high DMSP-producing taxa, significant nutrient starvation and oxidative stress.[15,18] Although DMS loss to the atmosphere is thought to be the result of leakage from biological–physical interactions,[4] much of the variability observed remains unexplained.[19] Here evidence is presented that DMS concentrations in the ocean are influenced by the availability of microbially derived vitamin B_{12}.

Vitamin B_{12}, also known as cobalamin, is increasingly considered a critical growth factor for eukaryotic phytoplankton and may have the potential to shape microbial community structure,[20–22] whereas other potential roles for this cobalt-containing organometallic micronutrient in marine environments have received little attention. Although B_{12} appears to be produced solely by select bacteria and archaea, phytoplankton require it for growth as they possess a B_{12}-dependent methionine synthase (MetH) that is essential for cellular C1 metabolism.[23] Some eukaryotic phytoplankton also have a B_{12}-independent methionine synthase (MetE) that is much less effective than MetH and is only expressed under B_{12} deprivation.[24,25]

As methionine is the precursor to DMSP and S-adenosyl methionine (SAM), which is also involved in DMSP biosynthesis,[26] B_{12} is implicated in the biosynthesis of this important compound. The sole published study to examine the effect of B_{12} limitation on intracellular DMSP suggested that the alleviation of B_{12} limitation may increase intracellular DMSP concentrations in phytoplankton.[27] In addition, there are many marine bacteria that require exogenous sources of B_{12} for methionine cycling and a range of other metabolic functions,[22] suggesting that there are additional microbial processes that have the potential to be influenced by vitamin availability.

Materials and methods

Study areas and water collection

Experiments were conducted in the Ross Sea during the Controls on Ross Sea Algal Community Structure expeditions (CORSACS I; austral summer 2005–2006, NBP0601 and CORSACS II; austral spring 2006, NBP0608), and in the Pacific Ocean on the Metalloenzymes (MetZyme) Cruise (KM1128; Fig. 1a). In the Ross Sea, water was collected from ~3-m depth using a trace-metal clean (TMC) Teflon diaphragm pumping system.[20] In the Pacific Ocean, water was collected from 40-m depth using TMC X-Niskin sampling bottles. All sampling and incubation bottles were detergent and acid cleaned (0.1 % citranox (Alconox, Inc., White Plains, NY, USA) for 48 h, 10 % hydrochloric acid (Baker Instra-analysed; Avantor Performance Materials, Center Valley, PA, USA) for 7 days and clean pH 2 water rinsed (Milli-Q water adjusted to pH 2 with the addition of hydrochloric acid)). Polycarbonate incubation bottles were filled from TMC mixing carboys and manipulated in a positive-pressure TMC environment constructed with laminar flow hoods and plastic sheeting to avoid trace metal contamination. Incubation water was protected from exposure to direct ambient sunlight during each water collection.

Trace nutrient supplementation

In all cases, iron was added as iron chloride ($FeCl_3$) (>99 %, Fluka; Sigma–Aldrich Corp, St Louis, MO, USA), in weakly acidified (pH 3, SeaStar Baseline hydrochloric acid; SeaStar Chemicals, Sidney, BC, Canada) Milli-Q water. Vitamin B_{12} (99 % plant-cell culture tested cyanocobalamin; Sigma, Sigma–Aldrich Corp.) was added as a 10^{-6} mol L^{-1} to $10^{-6.3}$ mol L^{-1} solution in Milli-Q water, purified for trace metals by passing through a column of 2–3 mL of prepared Chelex-100 beads (BioRad Laboratories Inc., Hercules, CA, USA).[20]

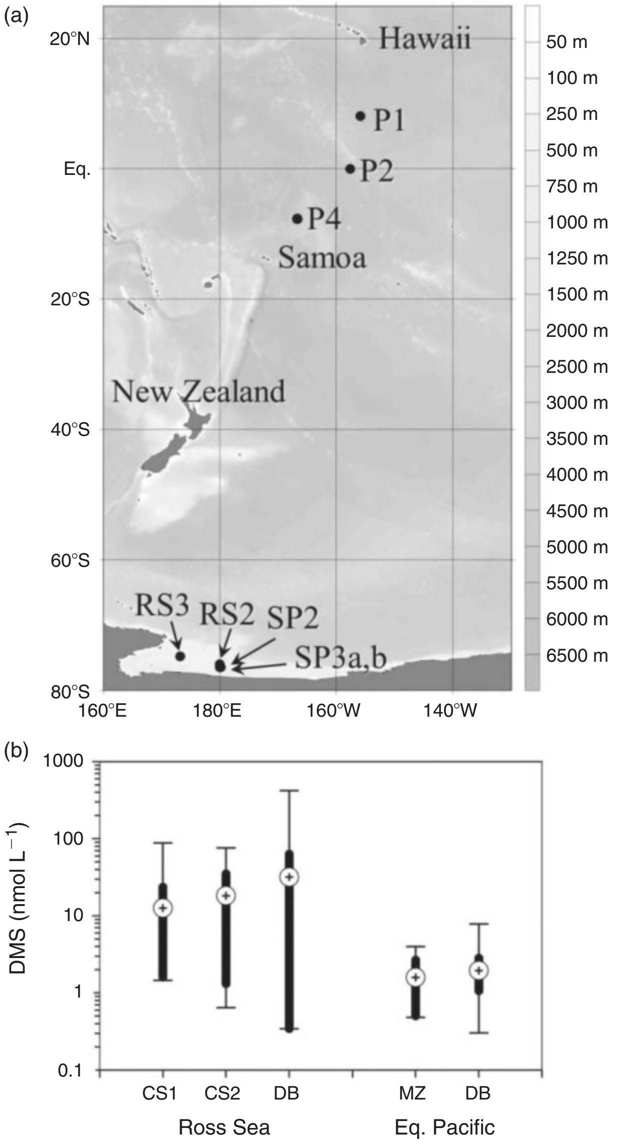

Fig. 1. (a) Locations of the experiments conducted in the equatorial Pacific Ocean (Sta. P1–P2, P4) and the Ross Sea (Sta. RS2, RS3, SP2, SP3a,b). Station coordinates for each experiment are given in the methods section. (b) Comparison of in situ sea-surface dimethylsulfide (DMS) concentrations measured in the area of each expedition. Data taken from the Global Seawater DMS Database[10] for the tropical Pacific Ocean and the Ross Sea. CS1, CORSACS1 expedition; CS2, CORSACS2 expedition; MZ, Metzyme expedition; DB, DMS Database. Average concentrations for each dataset are indicated by the crosses in the centre of the circles. The thick error bars represent one standard deviation. The thin whiskers represent the range of values observed.

Ross Sea incubation experiments: RS2, RS3

Incubation experiments using whole seawater with additions of 1 nmol L^{-1} added iron and 100 pmol L^{-1} added vitamin B_{12} (carried out in triplicate) were started in the Ross Sea on 08 January 2006 at 76.00°S, 179.98°E (RS2) and 16 January 2006 at 74.98°S, 173.33°E (RS3). These experiments were referred to as Incubations 2 and 3 in a previous publication.[20] Triplicate 1.1-L polycarbonate bottles were tightly capped and placed outdoors in deck-board flow-through incubators at ~20 % ambient light, shielded with neutral density screening (~120–260 μmol quanta $m^{-2} s^{-1}$). Ambient temperature (−2 to 1 °C) was maintained by a constant flow of surface seawater through the incubators. Samples were taken for DMS and DMSP concentrations at the beginning and the end of the experiments, which lasted 223 h (RS2) or 180 h (RS3). In both experiments, triplicate bottles were incubated per treatment and concentrations of DMS and DMSP were determined for each replicate at the final time point.

Ross Sea incubation experiment: SP2

Incubation experiments using whole seawater with triplicate additions of 1 nmol L^{-1} iron and 100 pmol L^{-1} vitamin B_{12} were started in the Ross Sea on 22 November 2006 at 77.50°S, 179.81°W. These experiments were conducted as described above, except that the bottles were incubated for 170 h in a shipboard laboratory van held at 1 ± 1 °C and a constant irradiance of ~160 μmol quanta $m^{-2} s^{-1}$ from blue fluorescent bulbs (Philips Electronics, 36W/18; 400–520-nm emission peak).

Ross Sea incubation experiment: SP3a,b

Bottle incubation experiments using whole seawater with triplicate additions of 1 nmol L^{-1} iron and 100 pmol L^{-1} vitamin B_{12} were started in the Ross Sea on 03 December 2006 at 76.50°S, 179.99°W. This station was referred to as Sta. SP3a in a previous publication,[27] in which ancillary data for this experiment can be found. This experiment was carried out for 124 h and incubated in a deckboard incubator as described for Sta. RS2 above (20 % ambient photosynthethically active radiation (PAR): 100–210 μmol quanta $m^{-2} s^{-1}$). A second experiment was conducted at this location (SP3b), prepared as described for SP3a but incubated in the laboratory van (as with SP2) rather than in the deckboard incubator.

Equatorial Pacific Ocean incubation experiments

In all cases, initial DMS concentrations were measured in quadruplicate for each experiment.

Equatorial Pacific Ocean incubation experiments: P1, P2

Two bottle incubation experiments using whole seawater with additions of 1 nmol L^{-1} added iron and 100 pmol L^{-1} added vitamin B_{12} were started at 8.09°N, 155.86°W on 10 October 2011 and at 0.04°S, 157.59°W on 14 October 2011. Whole seawater was dispensed into a 50-L TMC polypropylene mixing carboy and from there into 1.1 or 4.4-L polycarbonate bottles in duplicate or triplicate for each treatment (+Fe, $+B_{12}$, $+B_{12}$Fe and unamended control). The bottles were tightly capped and placed outdoors in deckboard flow-through incubators at ~20 % ambient light, shielded with neutral density screening (~300 μmol quanta $m^{-2} s^{-1}$). Ambient temperature (26 to 29 °C) was maintained by a constant flow of surface seawater through the incubators. At the beginning and the end of the experiments, samples were taken for DMS concentration analysis from the duplicate or triplicate bottles. P1 lasted for 77 h and P2 lasted for 48 h, ending in the middle of the light cycle.

Equatorial Pacific Ocean dose–response experiment: P4

An experiment was conducted at 12.00 S, 167.56 W on 21 October 2011 by filtering whole seawater through a 3-μm TMC polyether sulfone filter (to remove large phytoplankton; Sterlitech Corp., Kent, WA, USA) into 300-mL polycarbonate bottles. Quadruplicate bottles were supplemented with either 0, 5, 50, 200 or 1000 pmol L^{-1} B_{12} and after 2 days, DMS concentrations were measured.

DMS and DMSP analysis

Samples for DMS and $DMSP_d$ analysis on all three expeditions were collected following a small-volume gravity filtration procedure and analysed by cryogenic purge and trap gas chromatography techniques.[28,29] During the CORSACS expeditions, sample preparation was conducted in a cold-room held at 0 °C. A small aliquot (≤10 mL) of each sample was gravity-filtered through a Whatman GF/F filter, the filtrate collected and analysed immediately for DMS. A second aliquot (≤10 mL) of each sample was gravity-filtered through a Whatman GF/F filter, collected and acidified with 100 μL of 50 % sulfuric acid for the determination of $DMSP_d$. Care was taken when collecting the samples for DMS and $DMSP_d$ such that the filters and samples on the filters were not exposed to air to prevent cell lysis and the potential overestimation of $DMSP_d$.[30] A third unfiltered 10-mL aliquot was acidified with 100 μL of 50 % sulfuric acid for the measurement of total DMSP. Particulate DMSP was calculated as the difference between the total and dissolved DMSP fractions. During the MetZyme expedition, sample preparation was carried out at ambient laboratory temperature (~20 °C) and 20 mL of sample was filtered to offset lower analyte concentrations. All DMSP samples were stored at 4 °C for at least 24 h before analysis. Upon analysis, the DMSP samples were base-hydrolysed in strong alkali (2 mol L^{-1} sodium hydroxide) and analysed for DMS.

Instrumental analysis was carried out on a Hewlett–Packard 5890 Series II gas chromatograph (GC) during the CORSACS expeditions and a Shimadzu GC-2014 during the MetZyme expedition. Samples were sparged with ultra-high purity (UHP) helium (100 mL min^{-1} for 25 min). The gas stream was passed through a water trap (isopropyl alcohol held at −50 °C) to remove any water and then through liquid nitrogen to trap the DMS. The DMS trap was subsequently heated to 50 °C and the sample flushed into the GC at a flow rate of 20 mL min^{-1}. Chromatographic separation of DMS was achieved using a Supelco Chromosil 330 column (1.83 m packed × 3.18-mm outer diameter FEP Teflon) held at 40 °C. Both instruments were fitted with flame photometric detectors and the flow rates for flame gases (UHP hydrogen and Zero-Grade air) were set according to the manufacturer's guidelines for each instrument. Primary calibration of the instruments during each expedition was conducted using a Dynacal permeation tube (held at 30 °C; VICI-Metronics, Poulsbo, Washington) with daily calibration checks performed using base-hydrolysed DMSP standards (Research Plus, Inc., Bayonne, NJ, USA).

DMS data from Global Surface Seawater DMS Database

Data was taken from the Global Surface Seawater DMS Database (http://saga.pmel.noaa.gov/dms/, accessed 1 December 2012)[17] to generate the comparison presented in Fig. 1b. The selection criteria for DMS data for the central equatorial Pacific Ocean was defined as all data points falling within an area from 17°N, 154°W to 15°S, 174°W and for the Ross Sea as all data points falling within an area from 70°S, 164°E to 80°S, 164°W.

Statistical analyses

Student's unpaired *t*-tests were performed for statistical determination of differences where indicated. In cases where sufficient data points (i.e. $n > 2$) were available, 90 % confidence intervals were also assessed and plotted.

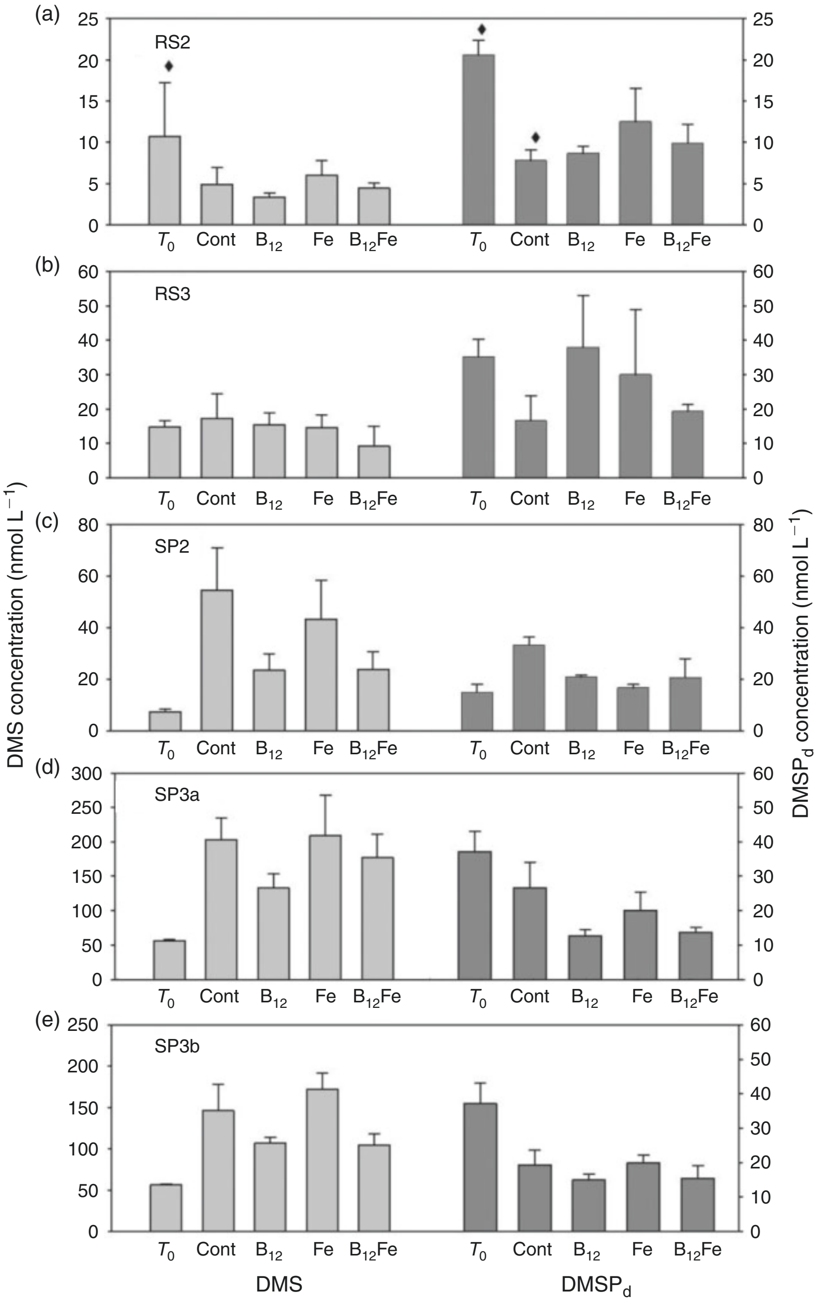

Fig. 2. Dissolved dimethylsulfoniopropionate ($DMSP_d$) and dimethylsulfide (DMS) concentrations from the Ross Sea B_{12} and iron addition ($+100$ pmol L^{-1} B_{12}; $+1$ nmol L^{-1} Fe) experiments. Concentrations at the beginning of the incubations (T_0) are shown for reference. (a) Sta. RS2. (b) Sta. RS3. (c) Sta. SP2. (d) Sta. SP3a. (e) Sta. SP3b. Errors bars represent the 90 % confidence interval unless denoted with a diamond symbol, in which case the standard deviation about the mean of duplicate treatments is presented.

Results

Here we describe measurements of DMS and $DMSP_d$ concentrations in multiple incubation experiments manipulating B_{12} and iron concentrations in the tropical Pacific Ocean and the Ross Sea (Fig. 1a). Considerable variation is seen in the levels of sea-surface DMS globally and these regions span the range of sea-surface DMS concentrations encountered in global oceans. The surface waters of the Ross Sea contain among the highest concentrations observed and the oligotrophic central equatorial Pacific Ocean among the lowest, with typically 1–2 orders of magnitude difference between the two (Fig. 1b).[17] The in situ levels of DMS measured during both CORSACS and Metzyme expeditions were well within the observed ranges and thus representative of these regions.

DMS and $DMSP_d$ concentrations at the beginning of each Ross Sea experiment (T_0) and the endpoints in the control, and $+B_{12}$, +Fe and $+B_{12}$+Fe treatments are shown in Fig. 2. Initial DMS concentrations ranged from <10 to >50 nmol L^{-1}, increasing over time in the springtime incubation experiments (SP2, SP3) and decreasing over time in the summertime

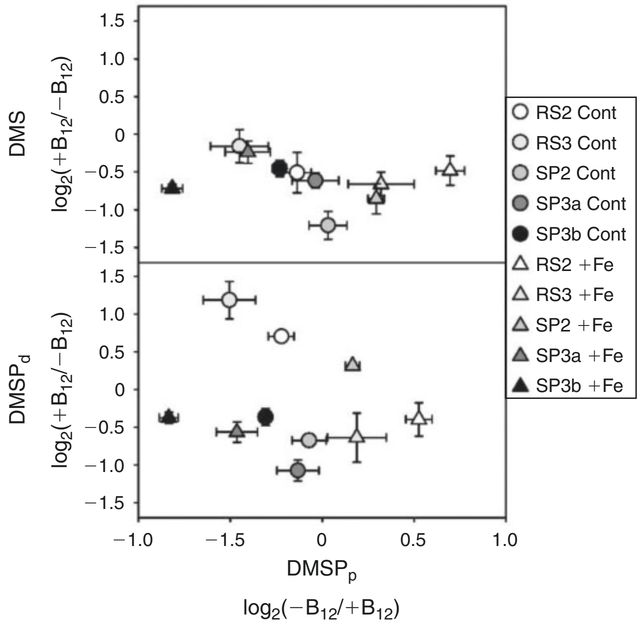

Fig. 3. $\mathrm{Log_2}$ fold changes in dimethylsulfide (DMS) (a) and dissolved dimethylsulfoniopropionate ($\mathrm{DMSP_d}$) (b) in treatments with and without added $\mathrm{B_{12}}$ at the end of each of the five Ross Sea incubation experiments plotted against $\log_2$ fold change in particulate DMSP ($\mathrm{DMSP_p}$) in the same experiment. Although the changes in DMS and $\mathrm{DMSP_d}$ are largely positive (lower concentrations upon $\mathrm{B_{12}}$ addition), there is no trend in $\mathrm{DMSP_p}$, suggesting that the observed decreases in DMS and $\mathrm{DMSP_d}$ with $\mathrm{B_{12}}$ addition are not driven by changes in $\mathrm{DMSP_p}$.

experiments (RS2, RS3). Despite this variability in magnitude of the change relative to initial values, DMS concentrations at the end of the incubations were typically lower in $\mathrm{B_{12}}$ additions than in controls or in incubations where only iron was added. Changes in $\mathrm{DMSP_d}$ were more variable, with $\mathrm{B_{12}}$ additions causing relative declines in final $\mathrm{DMSP_d}$ concentrations in only the springtime experiments (SP2, SP3). These changes in DMS and $\mathrm{DMSP_d}$ did not appear to be driven by changes in particulate DMSP ($\mathrm{DMSP_p}$) (Fig. 3). In only one instance did iron addition cause significant ($P < 0.15$) changes in DMS or $\mathrm{DMSP_d}$ concentrations relative to the control (Fig. 2c; SP2, DMS).

Incubation experiments conducted in the Equatorial Pacific showed similar trends; DMS concentrations were generally lower when $\mathrm{B_{12}}$ was added compared to unamended controls at the final time-point, regardless of whether the DMS concentrations increased or decreased from the beginning to end of the incubation experiment (Fig. 4). Iron did not cause significant ($P < 0.15$) changes in DMS concentrations in either P1 or P2. In experiments with larger phytoplankton (>3 µm) removed (Fig. 4c; P4), DMS concentrations also decreased with increasing $\mathrm{B_{12}}$ additions (Fig. 3c), although this was only significant ($P < 0.05$) at the highest $\mathrm{B_{12}}$ concentrations added. $\mathrm{DMSP_d}$ samples were also collected for the experiments P1 and P2. However, the loss of $\mathrm{DMSP_d}$ in the $+\mathrm{B_{12}}$ treatments was such that concentrations fell below the detection limit before the end of the experiments, making determination of the differences in $\mathrm{DMSP_d}$ at the end of the experiment in the control *v.* $\mathrm{B_{12}}$ amended treatments impossible.

Synthesising results from these two regions, DMS concentrations were significantly ($P < 0.15$) lower in $+\mathrm{B_{12}}$ treatments *v.* unamended controls at the end of five of seven incubation experiments (Fig. 5a). Overall, DMS concentrations were an average of 33.4 % (±15.1 %; 1 standard deviation) lower in the $\mathrm{B_{12}}$ treatments *v.* controls at the end of the incubation experiments. DMS concentrations were an average of 30.4 % (±10.4 %) lower at the end of the incubation experiments when $\mathrm{B_{12}}$ and iron were added relative to when iron was added alone; these changes were significant in two of seven experiments (Fig. 2a). Significant relative declines in $\mathrm{DMSP_d}$ with $\mathrm{B_{12}}$ supplementation were observed in only two of the five experiments (Fig. 5b); in one case the declines were significant in both iron supplemented and low iron treatments (SP3a). The length of each incubation varied according to the rate of macronutrient drawdown and was ended before macronutrients were depleted

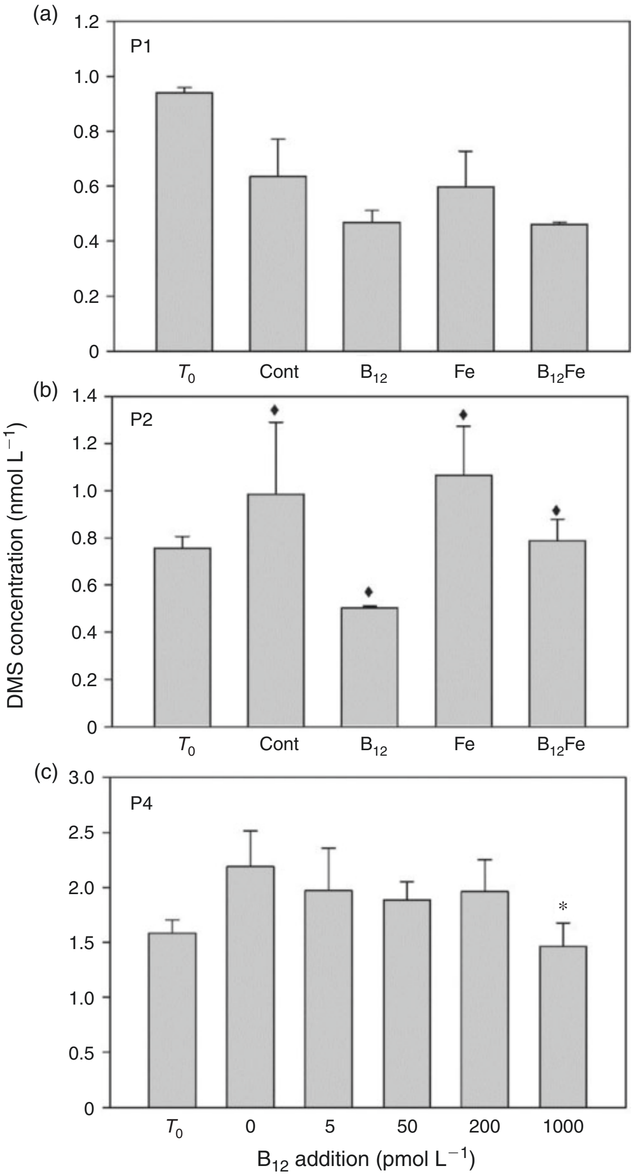

Fig. 4. Dimethylsulfide (DMS) concentrations from the equatorial Pacific Ocean $\mathrm{B_{12}}$ addition experiments. Concentrations at the beginning of the incubations (T_0) are shown for reference. DMS concentrations are shown for Sta. P1 (a) 72 h after the addition of 100 pmol $\mathrm{L^{-1}}$ $\mathrm{B_{12}}$ and 1 nmol $\mathrm{L^{-1}}$ Fe and for incubation P2 (b) 48 h after micronutrient addition. Panel (c) shows changes in DMS observed after 48 h in response to increasing doses of $\mathrm{B_{12}}$ in experiment P4. Errors bars represent the 90 % confidence interval unless denoted with a diamond symbol, in which case the standard deviation about the mean of duplicate treatments is presented.

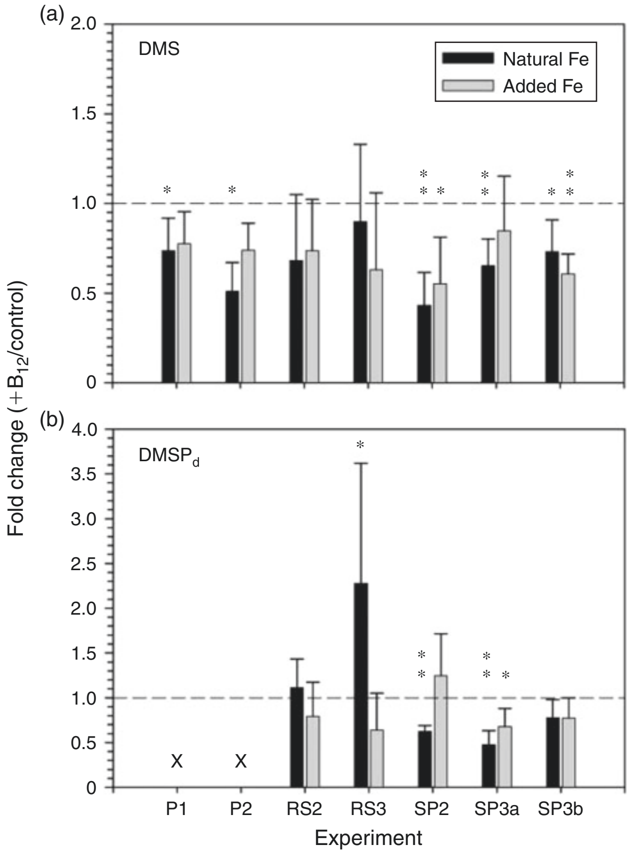

Fig. 5. Comparison of relative changes in dimethylsulfide (DMS) (a) and dissolved dimethylsulfoniopropionate ($DMSP_d$) (b) concentrations (relative to unamended controls) at the end of the incubations for communities containing ambient iron levels and for iron-supplemented communities. The horizontal dashed line at 1.0 on the *y*-axis represents no change (any number <1.0 represents a decline relative to the control). The error bars represent propagated standard deviations from duplicate or triplicate treatments. In experiments P1 and P2, $DMSP_d$ levels in the final time-point for all four treatments were below detection levels, so relative amounts of $DMSP_d$ could not be calculated (denoted with 'X'). One star symbol denotes that an unpaired *t*-test shows $+B_{12}$ treatments were significantly different ($P < 0.15$) from $-B_{12}$ controls and two stars indicate statistical difference with $P < 0.05$.

in the Fe-added treatments.[20,27] No trend in DMS concentration changes as a function of incubation length was observed across the seven experiments. Ross Sea incubation experiments were longer, as is typical for polar experiments. Only one time-point with measurements of DMS and DMSP are available for each experiment and thus only net changes can be assessed, not rates of gross changes in DMS or DMSP.

The decreases in final DMS and DMSP concentrations upon B_{12} addition were significant at the most stringent confidence interval in the springtime Ross Sea experiments (SP2, SP3), when the communities were dominated by *Phaeocystis antarctica* and where bacterial abundance was lower than in the diatom-dominated summertime communities (RS2, RS3).[20,27] The experiment with the smallest relative change in DMS and $DMSP_d$ concentrations with and without B_{12} addition was RS3 (Fig. 5). This was, interestingly, the experiment in which phytoplankton growth was co-limited by iron and B_{12} availability.[20] Phytoplankton growth was not B_{12} limited or co-limited in RS2, SP3 or SP2.[20,27]

Discussion

DMS concentrations were observed to decrease upon B_{12} addition relative to controls in five of seven incubation experiments across two different oceanic regimes. This trend emerged despite what is presumably considerable biological variability in B_{12} concentrations, the identity of primary producers, bacterial and archaeal community composition and a host of other factors. The experiments reported here were primarily designed to examine the availability of trace metals and B_{12} on phytoplankton communities.[20,27] Although bottle incubations such as these may, in some cases, be prone to artefacts that result from removing or altering physical factors that affect microbial growth and are not necessarily optimal for studying DMS dynamics, the meta analysis completed here allows for increased confidence in such experiments and highlights an intriguing trend. Moreover, although the changes are small, they are significant in several of the experiments conducted. The following, albeit largely speculative, discussion outlines several plausible explanations for the observations, helping reinforce the idea that vitamin B_{12} availability can affect the biogeochemical cycling of DMSP and DMS.

If the primary effect of B_{12} on microbial (algal and bacterial) sulfur cycling was through methionine synthase activity-induced changes in phytoplankton DMSP production, one might expect an increase in DMS upon B_{12} additions, rather than the decrease observed here. This decrease in DMS upon B_{12} addition (Fig. 5a) thus suggests that B_{12} availability is affecting additional downstream microbial sulfur transformations. Findings from the Ross Sea experiments also indicate that the observed changes were not caused by changes in cobalt concentrations. When cobalt was added, either alone or with iron, no changes in biomass (measured as chlorophyll-*a*) were observed.[20,27] DMSP and DMS were also measured in the cobalt addition experiments and no significant change was detected for either compound (data not shown).

There are at least four possible microbially based explanations for the observed decrease in DMS concentrations upon B_{12} addition (Fig. 6). The first involves B_{12} availability boosting the DMSP demethylation pathway and therefore reducing the pool of DMSP that gets cleaved to DMS. The only characterised DMSP demethylase (DmdA) has not been shown to require B_{12} and does not have a known B_{12}-binding site.[5,6] However, the reaction that it carries out, the transfer of a methyl group from DMSP to tetrahydrofolate (THF), could potentially be limited by THF availability. B_{12} deficiency in cells with MetH has been shown to lead to 5-methyl-THF build up and THF deprivation.[31] Thus, added B_{12} could enhance DMSP demethylation by recycling 5-methyl-THF to THF and boosting MetH activity.[32] This mechanism would only apply to bacteria or archaea utilising both DmdA and MetH, such as some *Roseobacters*, but potentially not to the SAR11 clade because cultured and sequenced representatives do not appear to possess methionine synthase.[33]

A second possibility is that there is an undescribed oceanic DMSP demethylase that performs the same reaction as DmdA but uses B_{12} as a methyl group carrier. Methyl transfer reactions can be much more efficient when B_{12} is used as the carrier, as seen in the methyl transfer conducted by MetH compared to MetE.[34,35] At present, DmdA has been found in 27 % of bacterial genomes in global ocean metagenomic analyses whereas the genes encoding enzymes for the next two steps in the DMSP demethylation pathway (*dmdB* and *dmdC*) have been found in 61 %,[16] leaving

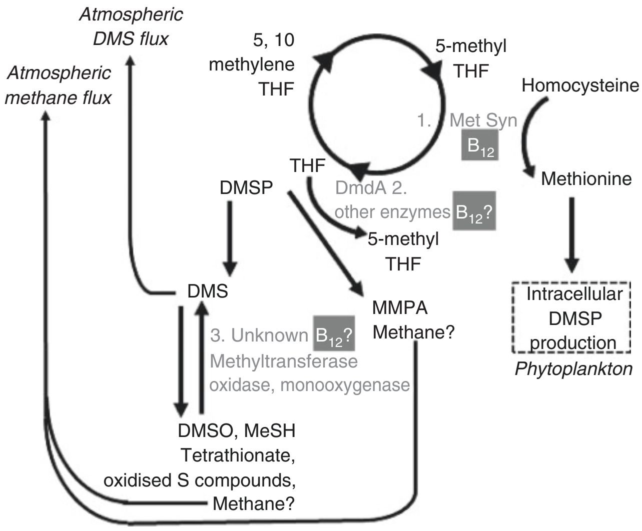

Fig. 6. Schematic representation of three possible mechanisms by which B_{12} availability may affect dimethylsulfide (DMS) concentrations. MMPA, methylmercaptopropionate; MeSH, methanethiol; DMSO, dimethyl sulfoxide. 1. Relieving B_{12} limitation of B_{12}-dependent methionine synthase (MetH) activity could alleviate tetrahydrofolate (THF) deprivation and increase dimethylsulfoniopropionate (DMSP) demethylation at the expense of DMS production by DMSP cleavage. 2. An alternative enzyme serves the same role as DMSP demethylase (DmdA) but utilises B_{12} as a methyl group carrier; under B_{12} stimulation this could lead to enhanced DMSP demethylation over cleavage. 3. DMS degradation could be dependent upon (as yet incompletely described) B_{12}-dependent methyltransferase enzymes. These possible mechanisms have the potential to influence atmospheric DMS and methane fluxes.

room for an alternative methyltransferase enzyme to serve the same role as DmdA. Similarly, recent metatranscriptomic analyses revealed that organisms with no known DMSP demethylation or cleavage genes respond to DMSP additions with significant transcriptional changes,[36] suggesting that currently undescribed enzymes and pathways, such as this potential B_{12}-dependent methyltransferase, may play important roles in oceanic DMSP degradation. Thus, by either enhancing THF availability to DmdA or by stimulating an alternative methyltransferase, B_{12} addition could enhance $DMSP_d$ loss by the demethylation pathway relative to the cleavage pathway, resulting in less DMS formation. It should be noted that this explanation is not entirely consistent with our observations because there are several cases where DMS concentrations decreased upon B_{12} addition but $DMSP_d$ concentrations did not.

A third possibility is that B_{12} is involved in DMS degradation. Although bacterial DMS consumption has long been recognised,[14] very little is known about the pathways and proteins involved.[6] There are several lines of evidence suggesting that B_{12} availability may play a role in DMS degradation. Early studies suggested that under aerobic conditions DMS was degraded by two distinct pathways, one involving a monooxygenase and oxidase whereas the other involves a methyltransferase.[37] This methyltransferase was found to be inhibited by chloroform, leading to the suggestion that it uses B_{12} as an intermediate methyl group carrier because chloroform addition is known to inhibit B_{12}-dependent but not B_{12}-independent methyltransferases.[32] Notably, a large amount of oceanic DMS degradation is inhibited by chloroform,[14,38] supporting the notion that oceanic DMS degradation may be B_{12}-dependent. However, other metabolic processes may be affected by chloroform additions, so further examination of this relationship is required.[38] It has been shown that DMS demethylation by methanogens involves a B_{12}-dependent methyltransferase.[39] In addition, other recent observations also support a relationship between B_{12} availability and DMS degradation in members of the *Methylophaga* genus.[40,41] These data all support the possibility that DMS degradation in the surface ocean could be influenced by B_{12} availability but it is not clear whether this degradation is the direct effect of altering B_{12} availability or the conversion of B_{12} into other structurally similar corrinoid or cobamide molecules.[42–44]

It is also possible that the overall activity of bacterial groups responsible for DMSP conversion into methylmercaptopropionate (MMPA) and for DMS degradation is limited by B_{12} availability, and by relieving this limitation, DMS concentrations decrease independent of a direct molecular connection between B_{12} availability and reduced sulfur metabolic processes. B_{12} limitation of marine bacterial growth has not been evaluated to date, so this possible explanation also warrants further consideration and experimental examination.

An additional possibility is that B_{12} is involved in the degradation of both DMSP and DMS by the mechanisms outlined above. If these processes are indeed B_{12}-dependent, it is thus possible that they are B_{12}-limited under circumstances where demand exceeds supply. This potential limitation is hypothesised to be dependent upon bacterial and archaeal B_{12} production rates, phytoplankton and bacterial vitamin uptake rates and the rate of photochemical decomposition.[27] Since it has been suggested that phytoplankton production of DMSP is a major regulator of oceanic DMS and DMSP production is higher under states of nutrient limitation,[4,15] the observed decrease in DMS upon B_{12} addition could have also resulted from relief from nutrient limitation. However, given that the Ross Sea community analysed at Sta. SP3 and RS2 were iron limited,[20,27] and iron additions did not result in decreased DMS but B_{12} additions did (Figs 3, 4), this explanation seems unlikely. In addition, no relationship between changes in particulate DMSP and DMS concentrations was observed in these experiments (Fig. 4), suggesting that changes in phytoplankton production of DMSP induced by changes in micronutrient availability are not responsible for the DMS response observed here.

Finally, there is convincing evidence that cob(II)alamin can serve as an effective superoxide dismutase.[45] In seawater (dissolved phase), B_{12} is not likely to be an important sink of dissolved superoxide.[46] Within marine microbes, there is more uncertainty. Based on the hypothesis that DMSP, DMS and their degradation products act as an antioxidant system,[18] it seems worth considering the idea that an increase in B_{12} availability could decrease cellular reliance on DMS and DMSP as antioxidants. In these experiments, because both increases and decreases in particulate DMSP, a key antioxidant, were observed upon B_{12} addition (Fig. 3), it appears unlikely that a reactive oxygen species (ROS) scavenging function for B_{12} could explain the majority of the observed DMS decrease seen here. Similarly, current data indicates that minimum cellular B_{12} quotas vary by at least four orders of magnitude and are some six orders of magnitude lower than DMSP concentrations,[47,48] also suggesting that B_{12} may not be an effective intracellular ROS sink. Until intracellular B_{12} concentrations and abundance relative to other antioxidants are better constrained, it remains unclear whether cobalamin could be an important intracellular sink for reactive oxygen.

Although the idea of a ‘bacterial switch’ that regulates DMS degradation and emission to the atmosphere is not new,[4] the cellular mechanisms controlling the switch have remained elusive.[4,14–16] The findings presented here provide plausible mechanisms that may contribute to microbial modulation of oceanic DMS. A schematic overview of the possible B_{12} related trace gas modulation mechanisms described here and their potential implications for global processes are given in Fig. 6. This influence of B_{12} availability on DMS may contribute to an explanation for the ‘summer DMS paradox’.[49] It has been recognised for several years that at low-to-mid latitudes, the highest DMS concentrations occur in summer whereas chlorophyll-*a* levels in the mixed layer peak in late winter and spring[49]; current DMS models do not consistently reproduce this feature.[15,19] Strikingly, the lowest dissolved B_{12} concentrations appear to occur in summer.[50,51] In addition, B_{12} limitation of phytoplankton growth and high rates of B_{12} uptake by picoplanktonic populations (<2 μm, including bacteria) also feature prominently during summer months.[21] As a consequence of the trend observed here, a summertime decline in B_{12} levels and elevated competition for B_{12} could increase the relative importance of DMSP cleavage over the demethylation pathways and a decrease in DMS degradation, potentially helping to explain the summer DMS paradox. In addition, if DMSP is an important precursor for methylotrophic methanogenesis in aerobic marine environments as has been recently proposed,[52] then B_{12} may also influence oceanic methane production (Fig. 6). In total, these results imply that vitamin B_{12} could play previously unrecognised or more significant roles in regulating fluxes of climatically relevant trace gases; roles that warrant further rigorous investigation.

Moreover, given the recent finding that expanding anoxia may increase DMS generation at the expense of methanethiol production,[53] it seems likely that global change may have significant effects on organic sulfur cycling by a range of mechanisms. Probing how changes in B_{12} availability may act synergistically with expanding anoxia to influence DMS is a promising area for future research, particularly given the importance of B_{12} in anoxic microbial metabolisms such as archaeal methanogenesis and bacterial acetogenesis.[54]

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