Editor feedback to author response: os-2017-32 Lizotte et al. 15 Sep 2017
Thanks for the detailed responses and track changed modifications in response to reviewers RC-1 and RC-2.

Further responses required: Please can you also respond to RC-2 points that you have not responded to.

Answer ML. I sincerely apologize. I'm not sure how these points were lost from the review. In any case, please accept my apologies.

L616: The papers by Tortell et al. that emphasize DMS increases across oceanic fronts should be cited.

Answer ML. Absolutely, they should. The following references from the Tortell group were added to this part of the discussion as well as in the list of citations: Asher et al. 2017, Nemcek et al. 2008, Tortell et al. 2005, Tortell and Long 2009. "The heightened biological activity in these regions (Llido et al., 2005) is thought to lead to intensified carbon drawdown on seasonal timescales (Metzl et al., 1999) as well as high concentrations of DMS (Asher et al., 2017; Holligan et al., 1987; Matrai et al., 1996; Nemcek et al., 2008; Tortell, 2005; Tortell and Long, 2009)."

L643-669: To what extent the 50-fold range in DMSPd consumption rate constants cannot be due to methodological uncertainties in either DMSPd concentrations or the 35S experiments? This range factor seems very large, and the turnover at station 5 seems super fast (turnover time 1 h). More so when there is no correlation to bacterial abundance or production. I agree that bulk bacterial production holds less potential to drive DMSPd consumption than taxon-specific production, but a critical view of uncertainties is warranted. By the way, the range of turnover times shown in Table 3 for the present study is 0.1-1.6 d – if the fastest was 1 h, this should read 0.05-1.6.

Answer ML. We understand the concerns put forward by the reviewer here. We certainly cannot exclude any methodological uncertainties and analytical limitations, as is the case in any type of experimental setup. In order to address this we further discuss the potential caveats associated with methodology (measurements of K_{DMSPd} and $DMSP_d$) and we calculate a factor of error propagation as the estimation of $DMSP_d$ consumption rates by multiplication of the $DMSP_d$ loss rate constants (k_{DMSPd}) with *in situ* $DMSP_d$ concentration carries a larger uncertainty. The error propagation was calculated by adding the relative uncertainties in quadrature (square root of the sum of squares).
Here are the changes (in bold) made to this part of the methodology (lines 327 and beyond):

"The measurement of the above variables allowed us to estimate $DMSP_d$ loss rate constants (k_{DMSPd}), **DMSPd turnover rates (or consumption rates) by multiplying values of k_{DMSPd} with *in situ* $DMSP_d$ concentration, and** rates of gross DMS

production from $DMSP_d$ by multiplying values of **$DMSP_d$ turnover rates with** DMS yields. **We calculated the propagation of uncertainty for rates that represent estimations based on other measured variables by adding the relative error of each variable in quadrature and expressing them as percentages. The uncertainty associated with estimates of $DMSP_d$ turnover rates and DMS production rates from $DMSP_d$ were on average 35% and 37%, respectively. Furthermore, we cannot rule out any bottle effects during incubation experiment, nor can we dismiss potential filtration artefacts related to the determination of $DMSP_d$ concentrations with which the derived estimates are based on. However all measurements were made following the best practices published and available at the time of sampling. Finally**, the microbial transformation rates of $DMSP_d$ measured during these incubations are considered to stem mostly from bacterial processes however phytoplankton-related processes cannot be totally excluded as low DMSP-producing phytoplankton and picophytoplankton have been shown to assimilate $DMSP_d$-sulfur (Malmstrom et al., 2005; Ruiz-González et al., 2011; Vila-Costa et al., 2006b).

Concerning the last part of the comment referring to values in Table 3: The values were rounded to 1 digit of precision for the purposes of uniformity in Table 3, thus transforming 0.05d to 0.1d as the lower limit within the range for this study. We agree that this makes a difference so the range of values presented in Table 3 were kept at 2 digits of precision to reflect the precise turnover time.

L699-703: The relationship between the DMSPd-to-DMS conversion efficiency and rates of bacterial leucine incorporation is intriguing. You claim this is because as bacteria increase their C incorporation, they do it by cleaving more DMSP to use its C. I am not persuaded by the argument. Bacteria also increase their S demand, when increasing C incorporation. Why not taking up DMSPd as both a C and a S source? From the subsequent arguments, should we understand that abundance of other labile C forms (and potentially org S forms), bacteria exhibited low DMSP assimilation rates and rather they cleaved quite a share of the available DMSPd? But DMS yields were not particularly high either. Please clarify your arguments. You could also invoke phycosphere-associated processes. In blooms like these there may be many bacteria closely associated to microalgae and therefore exposed to even higher concentrations of DMSP.

Answer ML. We provide further details in order to clarify our arguments by adding these lines (**in bold**) to section 5.4.3:

5.4.3 Microbial DMS yield and gross production of DMS from $DMSP_d$

Microbial DMS yields, the conversion efficiency of $DMSP_d$ into DMS, varied from 4 to 17% with an overall average of 11% across the entire study region, irrespective of water mass provenance and bloom association (Fig. 4a). Our results add to the mounting evidence that, as a whole, the span in endogenous proportions of $DMSP_d$ consumed by bacteria and cleaved into DMS is similar across various oceanic environments (see Table 3). A significant and positive relationship was found between rates of bacterial leucine incorporation and DMS yields in this study ($r_s = 0.84$, $p < 0.01$, $n = 8$). This relationship

suggests that as carbon incorporation for protein synthesis was heightened in the microbial communities, the proportional use of DMSP as a carbon source also increased, leading to higher $DMSP_d$-to-DMS conversion efficiencies (Table 2). Furthermore, prokaryotic protein synthesis, estimated by the bacterial incorporation of leucine (Kirchman et al., 1985), appeared to be significantly associated with the supply of $DMSP_d$ in this study ($r_s = 0.86$, $p < 0.01$, $n = 8$, Table 2). **With greater bacterial production rates of C, it is likely that bacterial production of S was also heightened in this study with potential modifications in assimilation efficiency of S from consumed ^{35}S-DMSP. A trend of increasing ^{35}S-DMSP assimilation yields concomitant with increased leucine incorporation rates was seen (data not shown) but the lack of statistical significance limits further interpretation of this tendency. The overall low proportion of ^{35}S-DMSP consumed and assimilated into macromolecules combined with the potentially rapid saturation of S requirements by the microbial assemblages, discussed previously, suggest that heterotrophic bacteria may have had access to ample sources of sulfur, including non-labeled *in situ* $DMSP_d$. High concentrations of both *in situ* $DMSP_d$ and $DMSP_t$ (Figure 2) indicate high accessibility for free-living (FL) bacteria of these methylated S compounds directly in the water column but also potentially for particle-associated (PA) bacteria in micro-zones surrounding phytoplankton cells and detrital particles such as faecal pellets and marine snow (see Review by Ramanan et al. 2016). These phycospheres and other micro-zones of enhanced gradients of dissolved organic matter (Amin et al. 2012, Bell and Mitchell 1972, Simon et al. 2002) are often associated with populations of bacteria that are distinct from the surrounding open habitat, that can vary according to phytoplankton community composition (Cooper and Smith 2015, Rieck et al. 2015), and that may possess higher uptake kinetics for substrates such as $DMSP_d$ (Scarratt et al. 2000). It cannot be excluded that such PA bacterioplankton were present in our experiment, in association with the DMSP-rich phytoplankton groups identified, leading to overall low S assimilation efficiencies from consumed ^{35}S-$DMSP_d$ despite changes in bacterial C production. This idea is supported by conclusions from Scarratt et al. (2000) suggesting that particle-associated bacteria can "afford" to make use of DMSP simply as a C source because their S requirements are amply satisfied.**

The fate of S in DMSP-metabolizing bacterial communities is complex and most likely affected by numerous factors, at least one of which is the S requirement relative to the availability of organic S. Findings from this study are consistent with the hypothesis that organic S in excess of bacterial requirements biases DMSP metabolism against demethylation (Kiene et al., 2000; Levasseur et al., 1996; Pinhassi et al., 2005). These observations agree with results from Lizotte et al. (2009) who observed an increase in DMS yields only following the addition of non-limiting concentrations of $DMSP_d$ and increases in microbial incorporation of leucine during an Ocean Iron Fertilization experiment in the Subarctic Pacific. Furthermore, at a physiological level, factors including bacterial carbon requirements and concentrations of DMSP degradation products can also exert an impact on the fate of DMSP (Kiene et al., 2000). Since the radioisotope technique used to examine the microbial cycling of $DMSP_d$ traces only the S moiety, significant respiration of C-DMSP can occur (Vila-Costa et al., 2010). As such, the combination of rather typical $DMSP_d$ turnover times (overall average of < 1 day) and

low DMSP-S assimilation efficiencies (< 5%) could be an indication of the availability of C **and S** rich compounds, including DMSP, to the bacterial assemblages in this study.

L778: Give range or std dev.
Answer ML. We added the std deviations in this part of the discussion and added the word "mean": "Coarse calculations that assume steady-state conditions suggest that transposing these net changes over a daily period amounts to a mean net community production of DMS from $DMSP_t$ of **15.2 ± 16.4 nmol L^{-1} d^{-1}** (n = 6) throughout the stations where data was available. This rough **mean** estimate is almost 3 times as high as the gross microbial production of DMS from $DMSP_d$ (average of **5.3 ± 9.9 nmol L^{-1} d^{-1}**, n = 6) in the same stations (sta. 3, 5, 6, 7, 8 and 9)."

L775-787: To support the idea that phytoplankton-mediated DMS production largely contributed to gross DMS production, note that, in the DISCO experiment, Steinke et al. (AME 2002) found that the majority of potential DMSP-lyase activity occurred in particles >10 m, namely dinoflagellates.

Answer ML. Although estimates of the potential lyase activity are difficult to transpose to the natural environment (because these rates are measured on extracted enzymes at saturating DMSP concentrations) we added the following phrase (and reference), in bold, to this section of the discussion: "The microbial DMS production rates from $DMSP_d$ in this study are also considerably lower than several of the community net production rates required to support microlayer DMS (range of -1445 to 5529 nmol L^{-1} h^{-1}) reported by Walker et al. (2016). **Estimates of the relative importance of phytoplankton-mediated DMS production are scarce, however a study conducted in waters of the North Atlantic during a summer coccolithophore bloom suggested that as much as 74% of the potential DMSP-lyase activity occurred in the > 10 µm particulate fraction, which contained a high proportion of dinoflagellates (Steinke et al., 2002).** Altogether our findings support the view that indirect and direct processes of phytoplankton-mediated DMS production were important contributors to standing stocks of DMS in the near-surface waters of the STF during austral summer."

RC-1, point 3 – suggest it is useful to reiterate here: Following "assimilation into bacterial biomass" with "and has not considered dissolved non-volatile degradation products."
Answer ML. We modified the phrase and added the following words (in bold): This study focused on two opposing short-term fates of DMSP-S following its uptake by microbial organisms: either its conversion into DMS, or its assimilation into bacterial biomass, **and has not considered dissolved non-volatile degradation products.**

RC-1, point 10 - The addition: "Dinoflagellate abundance was determined for surface waters (not for near surface waters) and is not shown here." is not particularly useful to the reader. Can a reference to data be given or numbers included in Table 1?

Answer ML. The phytoplankton data (including abundance and carbon content of dinoflagellates and other groups) will be addressed in a separate paper that is yet to be submitted. The following phrase was deleted: "Dinoflagellate abundance was determined for surface waters (not for near surface waters) and is not shown here.", and was changed (at lines 242-245) for "**No further information regarding the abundance of eukaryotic organisms in near surface waters is available however the abundance and carbon content of other groups of phytoplankton in surface waters will be discussed in a separate paper relating DMS cycling and marine biogeochemistry (*C. Law, personal comm.*)**."

RC-2 point 5 Suggest reword: "while the strength of the relationship between DMSPp and chl a is also strong (r2 = 0.57, data not shown)." With "while the correlation between DMSPp and chl a is of similar strength (r2 = 0.57, data not shown)."
Answer ML. We changed the wording of the phrase (at line 565), which now reads as follows: "A type II linear regression model suggests that 59% of the variance in pools of $DMSP_t$ can be explained by the variability in stocks of chl *a* (Fig. 5a), **while the correlation between $DMSP_p$ and chl *a* is of similar strength (r^2 = 0.57, data not shown)."**

RC-2 point 6 With the addition: "The SOAP blooms were coherent discrete areas of elevated ocean colour identified in satellite images characterised by a maximum of 1 mg/m3 chl a or higher. Sampling took place near the center of these blooms but also at stations on the periphery and outside the blooms (Table 1), as defined by the distance from the bloom centre and clear demarcation in surface biogeochemical variables (see Law et al., this issue)." I believe this should read: "The SOAP blooms were coherent discrete areas of elevated ocean colour identified in satellite images characterised by a minimum of 1 mg m-3 chl a or higher. Sampling took place near the center of these blooms but also at stations on the periphery and outside the blooms (Table 1), as defined by the distance from the bloom centre and clear demarcation in surface biogeochemical variables (see Law et al., this issue)." Saying blooms are chl-a areas up to 1 mg m-3 or greater sets no limits at all! I think this should read "by a minimum" rather than "by a maximum"

Answer ML. Yes absolutely, we changed the wording (at lines 217-222) as recommended (also including part of the next comment RC-2 point 7 just below): "The SOAP blooms were coherent discrete areas of elevated ocean colour identified in satellite images characterised by a minimum of 1 mg m^{-3} chl *a* or higher. Sampling took place near the center of these blooms but also at stations on the periphery and outside the blooms (Table 1), as defined by the distance from the bloom centre determined from pre-site surveys with bloom centre marked by a drifting spar buoy (see Law et al., this issue)."

RC-2 point 7 (and parts of 6) You say: "We are not certain what the reviewer is asking here. If possible, added information would help us address any concerns regarding this

part of the paper.“ I read that the reviewer is questioning the partitioning of sample sites between "in" the bloom and "in the vicinity" of the bloom and you do mention that this is a geographic distinction - Would it be more accurate to replace "and clear demarcation in surface biogeochemical variables (see Law et al..." with: “determined from pre-site surveys with bloom centre marked by drifting spar buoy (see Law et al...."” I read that the reviewer questions variables in Table 1 including Chl-a, nutrients and DMSP:Chla that do not show clear differences related to e.g. nutrient drawdown in bloom or greatly elevated Chla or DMSP in the bloom compared with the 2 stations north and south of blooms. (Perhaps this can be addressed by discussing that stations adjacent to bloom were also in generally productive waters).

Answer ML. Thank you very much for the precisions. As mentioned above (comment RC-2 point 6) we first changed the following phrase to complete information on the sampling strategy: “The SOAP blooms were coherent discrete areas of elevated ocean colour identified in satellite images characterised by a minimum of 1 mg m^{-3} chl *a* or higher. Sampling took place near the center of these blooms but also at stations on the periphery and outside the blooms (Table 1), as defined by the distance from the bloom centre **determined from pre-site surveys with bloom centre marked by a drifting spar buoy** (see Law et al., this issue).” We also added the following phrase to the methods section (line 222 and beyond): “**Note that stations adjacent to the blooms were also located in generally productive waters (Table 1)**.”

Additional corrections: I note error in footnote to Table 1 Change "then the 9 presented" to "than the 9 presented

Answer ML. Done, the word “then” was changed to “than”