Anonymous Referee #1

General Comments The authors report that there is a significant isoprene sink in the ocean, that needs to be accounted for to explain the observed concentrations of isoprene in the waters. In situ estimates of marine isoprene production is not made to the same extent of other biogenic hydrocarbons of global significance. Many of us still do not believe that marine isoprene is significant globally. It can be as little as 1 Tg per year if you accept conservative models, or some other more significant number, if you believe biologically meaningful assessment of empirical estimates. More studies such as the one by Booge et al will help us get closer to resolving this debate and help expand the research field of marine VOC-atmospheric interactions.

We thank referee #1 for reviewing this manuscript and for providing helpful comments. We will address the comments in the following (bold). The lines refer to the originally uploaded manuscript.

My biggest concern with this paper is the way in which the authors have assigned chlorophyll normalised isoprene emission rates to phytoplankton functional types *PFTs and also the emission factors derived from light response curves (tables 2 and 3, and the papers that are cited there). The authors themselves recognize clearly in the introduction and again in conclusion (L345-348, L480 onwards) that there are significant species-specific differences in isoprene emission capacities with respect to temperature (e.g. Exton et al. 2013) and light levels (e.g. Meskhidze et al 2015). Such studies are meaningful and important as individual studies. They may even provide a broad understanding of what a PFT does. There must be some caution while choosing species that are truly representative of a PFT while trying to derive an emission factor. Booge et al., have carefully left out species studied at subzero temperatures (which is a good thing as reflected in the table of Booge et al 2016 in ACPD (I have not read that paper fully). However, it is clear that they have included many species that are globally not relevant in terms of their abundance and those grown under different culture conditions. In those papers cited, cultures were grown at 16, 20 and 26 °C. SST is crucial for isoprene production. 10-degree increase can increase isoprene emission by 2 to 3 times over long term, and even higher levels over the short term in terrestrial ecosystems. E.g. In Table 3 of Exton et al (2013), they provide separate Pchlo for temperature and light response (irrespective of PFTs) and there are huge differences. Bonsang et al (2010) grew culture at a max light intensity of 100 umol/m2/s, Colomb et al (2008) did it at 250 umol/m2/s, Exton et al (2013), did measurements at 100 to 300 umol/m2/s. For all of these reasons I worry about the tenuous discussion on the Pchlo, and Pdirect presented in this paper.

- We absolutely understand the concerns of referee #1. The production rates of different PFTs vary depending on temperature and light intensity, which is also stated in the manuscript (l.61, l.376), and, in every case there will surely remain uncertainty when averaging over different species of one PFT. In the following we would like to respond to the points stated by referee #1.

1) Palmer and Shaw (2005) used bulk chl-a concentrations and a globally averaged production rate of 1.8 µmol (g chl-a)⁻¹ day⁻¹ in order to calculate the isoprene production in their model. In Booge et al. (2016) we could use actual isoprene field measurements in order to improve this model by a factor of ~10, using actual averaged PFT concentrations, rather than using bulk chl-a concentrations. The next step is now, as we tried in this paper,
to include the light dependency of the different PFTs to test if these rates from laboratory tests are somehow suitable for calculating isoprene concentrations in the ocean.

(2) We set our focus on the light dependency due to the natural cycle of light, which is applicable to the entire ocean. Laboratory studies (e.g. Exton et al., 2013; Shaw et al., 2003) could show that almost all isoprene is produced during daytime with higher production during higher light levels. This is also applicable to a depth profile in the ocean with higher light levels at the surface and lower light levels with depth in the mixed layer, if we assume the temperature constant.

(3) You correctly mentioned the different temperatures at which the laboratory studies were carried out, and referring to Table 3 of Exton et al. (2013), that the temperature, averaged over all different PFTs, has an influence on the isoprene production rate. But as these production rates are chl-a normalized rates it is worth to look at the rates dependent on chl-a, which is shown in Figure 3 in Exton et al. (2013):

![Figure 3](image-url)

Fig. 3. The relationship between Chl a concentration and isoprene production rate in laboratory phytoplankton cultures grown at three different temperatures, showing regressions for (A) strains grown at -1°C (filled circles), (B) strains grown at 16°C (open circles), and (C) strains grown at 26°C (filled triangles; regression equation values are shown in Table 3). Also shown is an overall regression for all strains at all temperatures (“pooled”), and the SST-independent relationship used in previous global models of marine isoprene identified by Shaw et al. (2003; 0.13 µmol isoprene [g Chl a]⁻¹ h⁻¹). Outlying results for Dunaliella tertiolecta are omitted from the regression equations displayed here.

During our studies the chl-a concentration ranged from 0.1 up to 8 µg L⁻¹, which is at the very low end of the chl-a concentration range shown in Exton et al. (2013), (see where the black triangles (experiments at 26°C) and open circles (experiments at 16°C) are overlapping each other (note: their x-axis unit is g L⁻¹)). The highest measured isoprene production rates were obtained at 26°C (top black triangles) which were in a chl-a regime that does not represent our study areas.

(4) By including the influence of light intensities only in this study, we were able to examine the temperature influence independently. We had the opportunity to corroborate the temperature-dependence found during laboratory studies directly in the field, since we did not include it from the beginning of our analysis.
Specific Comments L170 onwards and again L290 onwards: You say that haptophytes were the most dominant PFT in all three cruises (L330) and diatoms were dominant in coastal upwelling zone (figure s4). How do you explain fig s3, where haptophytes have very low emission response at light intensities <200 umol/m2/s, which is lower than that of diatoms. From your own figures (S1 and S2) light intensity below 10 m of the sea surface was less than 100 umol/m2/s. How can EF of haptophytes (L335) be greater than that of diatoms at the working light intensities in the ocean? Why use single point light response curves (figure s3) for cryptophytes and dinoflagellates? What species were used to obtain those curves in figure s3? See figure 1 of Gantt et al (2009, ACP). They have a light response curve that is based on measurements made at 4 or 5 different light intensities for each PFT and responses are strikingly different to what you are proposing. Why wasn’t their study considered in Table 2?

- We agree that the isoprene production rates of haptophytes at 45 µmol m⁻² s⁻¹ and 75 µmol m⁻² s⁻¹ (data from Shaw et al. (2003) and Bonsang et al. (2010)) are lower than the production rates for diatoms at the same light levels (data also from Shaw et al. (2003) and Bonsang et al. (2010)). At higher light levels (300 µmol m⁻² s⁻¹) the production rate of haptophytes is higher than for diatoms. In order to calculate the emission factor (EF) of each PFT, we applied a log squared relationship (following the approach of Gantt et al. (2009)). Therefore, in comparison to the individual measurements, the log squared curve is overestimating the production rate at lower light levels, but also underestimation the production rate at higher light levels. However, it is the best fit for all three data. The same is true for every PFT when applying the log squared fit. Even though this fit is associated with uncertainties depending on the individual data, the isoprene production rate for each PFT is still an average value of all investigated species within one PFT (as applied in Booge et al. (2016)), but now has a light dependency implemented, with significant influence on production rates.

To caution the reader, that there are uncertainties using a log squared fit, we added a sentence to line 300: “....2) uncertainty of using a light dependent log squared fit. Measurements from different laboratory studies used different species within one group of PFTs. All species within one PFT group were combined to produce a light dependent isoprene production rate (Figure S3), although the isoprene production variability of different species within one PFT group is quite high. This will certainly influence P_direct, but cannot explain the 70% difference between P_direct and P_need measured at SPACES/OASIS and ASTRA-OMZ (equator) (Figure 5);”

Figure S1 actually can lead to the conclusion that the light intensity below 10 m of the surface was less than 200 µmol m⁻² s⁻¹, but this is not true for most of the data. This data shown is an example from the SPACES cruise at approximately 25°S, which was the cruise operating in the highest latitudes of all three cruises. The mean light intensity was higher for all other data at lower latitudes near the equator (shown in updated Figure S1). The depth profiles of the stations chosen in Figure S2 from ASTRA-OMZ were performed during sunrise and sunset, resulting in lower light intensities.

We understand that this figure might lead to confusion as referee #1 stated, therefore, we changed figure S1 as follows: Instead of using one single day as an example, we calculated the total mean of all cruises of the hourly radiation measurements from the ship (Figure
S1a) and a total mean calculated PAR over the course of the day, depending on depth (Figure S1b). We also included the mean MLD of each cruise for a better understanding of the light levels present when sampling different depths.

We followed the approach of Gantt et al. (2009) to determine our light dependent production rates in the original publication (and continue to do so here). Only one production rate was available in the literature for cryptophytes, dinoflagellates, and *Prochlorococcus*. For haptophytes and diatoms, we used 3 and 6 light dependent isoprene production rates, respectively, and assuming a similar log squared dependence, as Gantt et al. (2009) did for *Prochlorococcus* and *Synechococcus* in their study.

We added a footnote to Table 2 stating that the specific species of tested PFTs can be found in the cited literature.

We did not add the laboratory derived production rates of Gantt et al. (2009) to our calculation because they only provide the EF and not the actual rates. Moreover, we do not understand how Gantt et al. (2009) calculated their emission rates (y-axis, Figure 1 in Gantt et al. (2009)). It seems they calculated the EF of *Prochlorococcus* by converting an isoprene production rate of 1.5 µmol (g chl-a)⁻¹ day⁻¹ derived by Shaw et al. (2003) at 90 µmol m⁻² s⁻¹ to an hourly rate, and applied a similar log squared fit as observed for diatoms and coccolithophores. Shaw et al. (2003) used a 14 hours light cycle resulting in an hourly production rate of 0.11 µmol (g chl-a)⁻¹. When using their fit, you should expect a production rate of 0.11 µmol (g chl-a)⁻¹ h⁻¹, when using a light intensity of 90 µmol m⁻² s⁻¹. However, according to the figure 1 in Gantt et al. (2009), a production rate of 0.7 µmol (g chl-a)⁻¹ h⁻¹ is obtained at a light level of 90 µmol m⁻² s⁻¹. Gantt et al. (2009) report similarly high rates for all measured PFTs in their study. As an example, their measured isoprene production rates for diatoms (see blue line, Fig. 1 in Gantt et al. (2009)) are in the range of 1.3 and 1.8 µmol (g chl-a)⁻¹ h⁻¹ at light levels of about 350 and 750 µmol m⁻² s⁻¹, respectively. In comparison, the isoprene production rates from literature values we used for diatoms at light levels of 300-900 µmol m⁻² s⁻¹ were in the range of ~0.22 µmol (g chl-a)⁻¹ h⁻¹ (see Figure S3). Due to these big discrepancies, the EF of Gantt et al. (2009) is also strikingly higher than ours. We do not know how to resolve these differences, however we checked our calculations and cannot find an error. Therefore, we only used the approach, but not the data from Gantt et al. (2009) in our study.

L185 and L288 onwards: The big difference between $P_{\text{needed}}$ and $P_{\text{direct}}$ is most likely due to the way you have calculated Pchloro, since $P_{\text{direct}}$ is largely dependent on EF (which is highly sensitive to temperature, light intensity, and species distribution). You rightly identify this as a potential reason (L300) but as highlighted earlier, the justification is difficult. In the equatorial region $P_{\text{direct}}$ is lower than $P_{\text{needed}}$ (figure 5) because of high SST and possibly also due to low emission factor you are assigning to cyanobacteria. The discrepancy in diatoms dominated coastal waters during ASTRAOMZ is noteworthy. The spike in isoprene in site 14 and 15 correlates with diatom blooms in coastal upwelling zone. But, chlorophyll normalised emission suggests an overestimation of $P_{\text{direct}}$ in coastal sites. Isoprene is mixed quickly in MLD (as you rightly say in L265), hence no vertical trend above MLD. But, what about the relative contribution of phytoplankton below and above MLD to isoprene? Since the mixed layer is very shallow in coastal sites (figure 4d), is it possible that a large proportion of isoprene is locked below MLD? You do mention advective mixing in the thermocline being a slow process (L444). If you know phytoplankton abundances below and above MLD (likely also a function of plankton size), it is perhaps possible to understand this. Can this hold for the entire cruise, given
that MLD generally was lower here compared to SPACES- OASIS? You also have a significant proportion of chlorophytes in these waters (figure S5) and they don’t emit isoprene at high rates. What was their light response like?

- Yes, the referee is absolutely right, in equatorial regions \( P_{\text{direct}} \) is lower than \( P_{\text{need}} \) due to high SST. The temperature dependence of isoprene production rates is not included in the calculation of \( P_{\text{direct}} \). As stated in our first comment (point (4)), this was exactly what we wanted to test, if the temperature dependence can be seen in field studies.

Also, the initial production rate of cyanobacteria might be a reason that \( P_{\text{direct}} \) is significantly smaller than \( P_{\text{need}} \). This is what we could prove, when using our data to calculate new production rates \( (P_{\text{chloro}}) \) using the multiple linear regression. As shown in Table 3, the new \( P_{\text{chloro}} \) values for cyanobacteria are 2 to 7 times higher for OASIS and SPACES, respectively.

Yes, the referee is right, in contrast to the equatorial and open ocean regions, \( P_{\text{direct}} \) is overestimated compared to \( P_{\text{need}} \). In these coastal upwelling areas, diatom concentrations are highly elevated and are accounting for ~80% of all PFTs. We attributed this either to a missing sink in this upwelling area or to incorrect literature derived \( P_{\text{chloro}} \) values of diatoms (lines 293-304). The newly calculated \( P_{\text{chloro}} \) values for diatoms show that only a production rate of 0.5-0.6 instead of 2.5 µmol (g chl-a)^{-1} day^{-1} (Table 3) is needed to be in better agreement with \( P_{\text{need}} \) (Figure 5b).

Advection mixing in the thermocline is a very slow process. If there is a strong concentration gradient with high concentrations of isoprene slightly below the mixed layer, this process might contribute to some point to these concentrations in the MLD. We checked the individual stations but concentrations are not higher below the MLD. This can also be seen in Figure 4, looking at the mean depth profiles.

Light responses of all PFTs are shown in updated Figure S3. Thank you for pointing out this missing information.

L272-274: What you say in L280-284 is more appropriate than what you say here. Most of the previous studies have shown positive correlation between chl-a and isoprene concentration (as Table 1 shows) in the oceans. The role of SST is also pretty well established.

- Yes, again we agree with the referee that many previous studies could show a positive correlation between chl-a and isoprene. The trend is clear: the more chl-a, the higher the isoprene concentration; the higher the SST, the higher the isoprene concentration (to some extent). However, we wanted to point out that, despite this large scale trend, the relationships between chl-a (or SST) and isoprene for each study or subset of a study are not consistent (i.e. no unique regression equation which can adequately describe the correlation between chl-a and isoprene globally, shown in Figure A). For clarification we changed the sentence in line 272 to: “...it can be seen that, even if the correlations for most of the datasets are significant, there is no globally unique regression factor to adequately describe the relationship between chl-a (and SST) and isoprene.”
Figure A: Visualization of all significant regression equations of relationships between isoprene and chl-a in different oceanic regions (data taken from Table 1 in the manuscript). Dotted line: SST<20°C, dashed line: SST>20°C, solid line: no SST bin.

L280-284: There is strong correlation between SST, chl-a, and isoprene concentration in cooler waters during both SPACES and ASTRA-OMZ cruises. (summarised in Table 1). Easy to see also in figures 3 and 7, but not mentioned. The relationship seems to breakdown at temperatures >25 deg C. Why? The discussion on relationships between chl-a, SST and isoprene is not satisfactory.

- The lowest SST measured during SPACES was higher than 18°C, meaning that data from SPACES is not included in the regression equation with the strong correlation (R²=0.89, Table 1) which we think you are referring to. We mention the correlation of P\text{norm} and ocean temperature during ASTRA-OMZ (shown in Figure 7) in lines 401 and 402: “...the P\text{norm} values were lower (< 8 pmol (µg PFT)^{-1} day^{-1}) correlating with lower ocean temperatures.” However this is a correlation between isoprene and P\text{norm} and not chl-a. If we look at temperatures lower than 25°C, Figure 3 might suggest, that there is a strong correlation (especially for SPACES) between chl-a, SST and isoprene but in fact the correlation coefficient of R²=0.42 is not as high as one would expect from looking at the figure. Using the data from all 3 cruises the correlation is significant for both cases, >25°C and <25°C, but the correlation using temperatures <25°C (R²=0.37) is not significantly higher than using temperatures >25°C (R²=0.32). Therefore, a possible breakdown of a suggested relationship between chl-a, SST, and isoprene at temperatures >25°C is mathematically not proven. A breakdown of isoprene production rates of diatoms (and haptophytes) at temperatures >26°C is discussed in paragraph 3.4, lines 377-382.

L379: “Higher temperatures caused a decrease in isoprene production rate [in diatoms]. ...If this temperature dependence can be transferred from diatoms also to haptophytes...” Yes. surely to Emiliania but perhaps to not all haptophytes. Please cite Heurtas et al. 2011 (Proc B) and a more recent meta-analysis from Chen, 2015 (J Phyt Res). However, I must point out that the discussion on cyanobacteria and Prochlorococcus is not satisfactory. Together they are 40% of the total biomass
during SPACES-OASIS. They emit isoprene at high rates and considering how abundant they are, how tolerant they are even to temperatures >30 degC, they are really important to this discussion.

We added the references in the updated sentence starting in line 380: “Increasing ocean temperatures influence the growth rate of phytoplankton generally, but also differently within a group of PFTs. For haptophytes, Huertas et al. (2011) show that two strains of Emiliania huxleyi were not tolerant to a temperature increase from 22°C to 30°C, whereas Isochrysis galbana could adapt to the increased temperature. In general, the optimal growth rate temperature decreases with higher latitude (Chen, 2015), but the link between growth rate of phytoplankton and isoprene production rate is still not known. Assuming this temperature dependence can be transferred…”

We concentrated on the discussion of haptophytes, because this was the only PFT of the three most abundant PFTs that recurred during all three campaigns. Nonetheless, we can see the referee’s point that we have to add the results for cyanobacteria and Prochlorococcus to the discussion. Therefore, we added a paragraph starting at line 368: “Prochlorococcus was one of the three most abundant PFTs during SPACES and OASIS, but concentrations decrease to almost zero in the colder open ocean and upwelling regions of ASTRA-OMZ (Figure 1), which confirms the general knowledge that Prochlorococcus is absent at temperatures <15°C (Johnson et al., 2006). Our newly derived production rates confirm the actual laboratory derived rates, demonstrating Prochlorococcus as a minor contributor to isoprene concentration. However, Prochlorococcus is especially abundant at high ocean temperatures, where isoprene production rates from the other PFTs show evidence of decreasing. Cyanobacteria concentrations (excluding Prochlorococcus) were also related to temperature, but in contrast to Prochlorococcus, cyanobacteria were still abundant in colder waters during ASTRA-OMZ. The different derived isoprene productions rates for SPACES and OASIS might be related to the different mean ocean temperature and light levels during these cruises. During SPACES, with lower ocean temperatures and lower light levels, compared to OASIS, the production rate is higher. This relationship would confirm the findings of two independent laboratory studies of Bonsang et al. (2010) and Shaw et al. (2003). Bonsang et al. (2010) tested two species of cyanobacteria of at 20°C and found higher isoprene production rates than a different species tested by Shaw et al. (2003) at 23°C and even stronger light intensities. However, Exton et al. (2013) measured the same rate as Shaw et al. (2003) at 26°C for one species, but a 5-times higher production rate for another species at the same temperature. This leads to the conclusion that the production rate is not dependent on one environmental parameter and varies from species to species within the group of cyanobacteria.”
Haptophytes are the biggest consumers of bacteria in the ocean (please cite Unrein et al. 2013, ISME J). Now, please reassess figure 9.

- Figure 9 shows that at stations where isoprene concentrations are elevated, bacteria cell counts are elevated, too. We do not know if these two parameters are linked primarily to each other but we considered that there might be a correlation of bacterial cell counts and isoprene concentration due to the abundance of isoprene attracting bacteria to feed and thrive. If there is a lot of isoprene to eat (e.g. energy source), the bacteria abundance could increase, independent of any phytoplankton influence. This would support the findings from Acuña Alvarez et al. (2009) who showed that isoprene production by phytoplankton could facilitate the amount of hydrocarbon-degrading bacteria. However, due to the relatively high production rate of haptophytes in comparison to the rate of bacterial consumption of isoprene, we hypothesized that this correlation could not be seen anymore when haptophytes were dominant (>33%).

We gratefully acknowledge the information that haptophytes are important grazers of bacteria. This helps to explain our results (Figure 9) in a more reasonable way. We added the explanation for Figure 9 starting at line 459: ”This is a high isoprene production rate and we could assume higher isoprene concentrations with higher concentrations of haptophytes. This relationship, however, is not evident (data not shown), which may be attributable to other processes masking this relationship. Multiplying the chl-a normalized isoprene production rate of 17.9 µmol (g chl-a)⁻¹ day⁻¹ with the chl-a concentration of haptophytes results in a mean isoprene production rate of ~ 3 pmol L⁻¹ day⁻¹, which is about 4 times higher than the mean calculated loss rate due to bacterial degradation over all cruises (~ 0.8 pmol L⁻¹ day⁻¹). This could hide the correlation of isoprene concentrations with bacteria when haptophytes are dominant (>33%). In addition, haptophytes themselves are suggested to be the main marine bacterial grazers, compared to other PFTs (Unrein et al., 2014). This leads to the hypothesis that, if there is a lot of isoprene that can be used (e.g. as energy source) by bacteria, also the bacteria abundance will increase, independent of any PFT. However, if the phytoplankton community is dominated (>33%) by haptophytes, the isoprene concentration is no longer correlated to the bacteria abundance, due to the grazing of bacteria by haptophytes (Fehler! Verweisquelle konnte nicht gefunden werden., total bacteria cell counts of black points are lower than of the red points at similar isoprene concentrations).”

L487-490: “The results show that the isoprene production is influenced by light, ocean temperature, and salinity, with an indication that the nutrient regime might exert some influence”. The same point has been made more elaborately by others (please cite Loreto and Dani 2017, Trends Plant Sci), where PFTs, temperature, nutrients and their impact on isoprene is anticipated including the parallel you seem to draw between dimethylsulphoniopropionate and isoprene (L385).

- We thank the referee for pointing out this additional reference. We would like to make sure, however, that within our manuscript we focus on the production rate of isoprene.

We changed the sentence starting line at 488 and added a second statement focussing on the actual rate of isoprene production: “The results confirm findings from previous laboratory studies that the isoprene production is influenced by light and ocean temperature, due to stress, and nutrients, due to their effect on changing phytoplankton communities and their abundances (e.g. Dani and Loreto, 2017;Shaw et al., 2010).
Moreover, our data leads to the conclusion that isoprene production rates in the field, irrespective of phytoplankton communities and their abundance, are influenced by salinity and nutrient levels, which has never been shown before.”

Technical comments: L52-54: It is one thing finding extraordinary numbers and then it is quite another explaining how and why? Your own estimates are closer to what we know from other marine waters.

- We absolutely agree. However, in the introduction section of the manuscript we tried to give an overview about the current knowledge/findings related to the biogeochemical cycling of isoprene. As there are not many oceanic isoprene studies published, it is worth to give an overview about the concentration range of marine isoprene concentration, which also includes those publications with extraordinary numbers. We do not try to explain why those numbers are so high, but rather just present them as published results.

L170: “Isoprene production rates of different PFTs were determined in laboratory phytoplankton culture experiments (see Table 2 in Booge et al. (2016))”. The measurements listed in the original table are also sourced from literature. Please state the same.

- We changed the sentence to: “…were determined in laboratory phytoplankton culture experiments (see a collection of literature values: Table 2 in Booge et al. (2016)) and…”

Figure 6: Missing letters a, b, c, d in subfigures

- Done. Thank you for pointing that out.

Figure S4: In a few sites, the category of others is really big. ?

- We think that the referee is referring to Figure S5, not S4. Yes, we agree that the proportion of “others” of the total phytoplankton chl-a concentration e.g. at station 1 during ASTRA-OMZ is 50%. Firstly, this is an exception and, secondly, “others” consists of several PFTs (i.e. 5 different PFTs). We tested and found that using the whole community for the calculations does not lead to different results in production rate and, furthermore, in some cases, to highly unlikely production rates for the less abundant PFTs. Therefore, we would like to keep our evaluation with the most abundant 3 PFTs.

References


