Response to Referee #3 (our replies are in bold)

The manuscript by Ashley et al. proposes the use of the carbon isotope composition of selected fatty acids present in sediments as a palaeoproductivity proxy in an Antarctic polynya environment (Adélie Land). The topic, totally in line with the journal Biogeosciences, is worth being investigated as proxys of paleoproductivity, especially in Polar Regions, are still lacking. The authors present an interesting set of quantitative and isotopic data, and based on their expertise in polar environments, discuss their possible significance in terms of biogeochemical changes recorded in sediments. The approach is interesting but the discussion and the conclusions raised by the authors may appear a little over-optimistic as many assumptions are made and some potential biases are discarded too easily and/or overlooked. There are a number of issues that the authors should take into consideration before the manuscript can be considered for publication. Comments are made chronologically, regardless of their importance.

Line 89 and manuscript throughout: It should be made clear in the manuscript that the data are based solely on free FA which represent only part of the total FA present in sediments (especially in modern to sub-recent sediments). If the selected FA indeed represent tracers of primary production, than it would be worth having a look at the bound (esterified) FA as well.

We thank the reviewer for pointing this out. The focus of this paper is on free, saturated fatty acids, which is normal practice within palaeoceanography (due to their better preservation). In our final submission we will make it clearer.

Lines 90-91: Please give more details on the use of BF3/MeOH as this treatment is known to be deleterious for some (poly)unsaturated FA.

In our final submission we will add some more details about the use of BF3-MeOH. It is true that Morrisson (1964, p.605) and Klopfenstein (1971) seem to suggest that high concentrations (around ~50%) of BF3 can lead to some loss of unsaturated FAs. However, since we used a concentration of 14%, this should not be an issue for our samples. Furthermore, as the focus of this paper is on saturated fatty acids, a loss of polyunsaturated fatty acids would not affect our data.

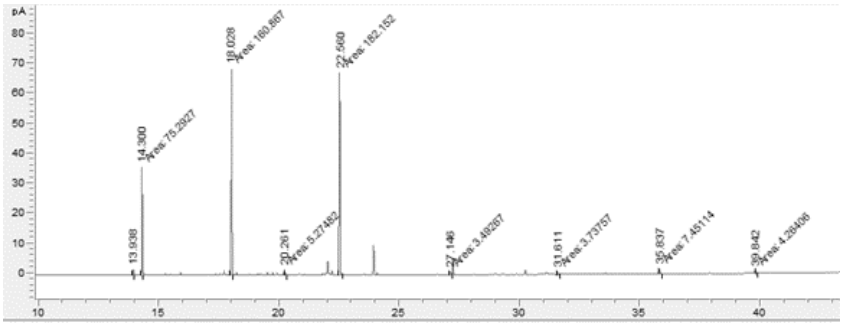
Lines 91-94 and Fig. S2. Please give more detail on the chromatographic conditions used (for both GC and GC-MS analyses) and refer to figure S2.

We thank the reviewer for pointing out this omission. In our final submission we will add details of the chromatographic conditions used for GC-FID and GC-IRMS analysis, including the GC column dimensions, carrier gas and oven temperature programme.

Also, the quality of the GC trace shown in figure S2 must be improved as, at such, a clear absence of unsaturated FA (which elute very close to saturated FA) is difficult to admit. As the authors know, the quality of compound specific 13C analyses is highly dependent on the purity of the compounds investigated and the absence of co-elution. Unsaturated FA often exhibit distinct 13C compositions compared to their saturated counterparts, so even small co-elution may significantly bias ïA¸d'13C values of saturated FA. An additional purification step using Si/Ag+ column chromatography may have been worth being investigated.

It is not too clear what the reviewer expects in terms of a better GC trace. Any unsaturated FAs were below the detection limit of the GC and thus did not show up in any GC traces, hence their absence in figure S2. We agree that any coelution of unsaturated compounds would affect the d13C values, but we carefully checked the baseline of samples during analysis and can confirm

that any coelution of other peaks was minimal. In our final submission we will include the following GC trace which may be slightly clearer.



Lastly, the peak attributed to the internal standard (C19 alkane) in Fig S2 is in fact most probably the C14 FA as it is not possible that the C19 FA elutes 15 minutes later than the C19 alkane. Please check peaks assignment (including the IS).

We are quite confused as to why the reviewer believes our peak assignment is wrong, without even knowing the chromatographic conditions used (which they rightly point out in their previous comment). It certainly is possible that the C19 FA could elute 15 minutes later than the C19 alkane on a slow oven programme. The C19 alkane was prepared in a hexane solution and analysed on the GC to determine its retention time prior to its addition into the FAME samples, giving us confidence in the position of the internal standard. Furthermore, a selection of samples was analysed before and after addition of the C19 standard which also made it clear which peak was the C19. GC-MS analysis of a selection of samples indicated that the C14 fatty acid was either very weak or absent. In our final submission, we will include the GC trace noted above which shows stronger peaks and here a very small C14 fatty acid peak is visible (at 13.9 minutes) next to the C19 alkane (at 14.3 minutes) indicating that they are in fact different peaks.

Lines 94-97. In line with the previous comment, more detail is undoubtedly required concerning CSIA. Which type of GC and conditions were used including the characteristics of the capillary column, the temperature of the interface and the oven, etc?

In our final submission we will add details of the chromatographic conditions used for GC-FID and GC-IRMS analysis, including the GC column dimensions, carrier gas and oven temperature programme.

Does 'Duplicate measures' means that each sample was analyzed twice?

Yes

If so, the error given is a min-max and not a standard deviation.

We will change the wording and remove the use of standard deviation here.

Were the measured ïA¸d'13C values corrected 1) for the methyl group added through derivatization

Yes the carbon isotopes were corrected for the addition of the methyl group due to derivatization and we will add details of this correction in our final submission.

and 2) for instrument deviation using a standard mixture?

No, the d13C values were not corrected for the instrument deviation, but this was monitored throughout analysis using external standards (F8, Indiana) and remained low throughout.

Are the stable isotope ratios expressed relative to the standard Vienna Pee Dee Belemnite (V-PDB)?

Yes, they are expressed relative to VPDB. We will include mention of this in our final submission.

Line 102: which IS were used for HBI?

To measure the HBI concentrations, we added 7 hexyl nonadecane (m/z 266) as an internal standard during the first extraction steps, following the Belt et al (2007) and Massé et al. (2011) protocols. We will include these details in the methods for our final submission.

Line 106: This is unclear as it sounds like a repetition of the previous sentence.

We will refine these sentences in our final submission.

Lines 125-126: The sole presence of saturated FA in (sub)actual sediments of (hyper) productive areas is very unusual (this is an additional reason why a very clear GC trace is needed in Fig. S2 which could even be included in the main manuscript). Would it be possible that unsaturated FA were (partly) destroyed by the BF3 treatment?

See lines 211-233. Here we discuss the breakdown of unsaturated and short-chain fatty acids in the water column and on the sea floor before burial. This most likely explains the sole presence of saturated FAs in our samples (Haddad et al., 1992; Matsuda, 1978; Colombo et al., 1997). Our understanding is that this is not unusual, unless the reviewer would like to share any specific references on this. The hyper-productive environment offshore Adélie Land is unique and not well studied thus it is hard to know how it compares to other sites. Although further investigations are certainly needed, we think it is unlikely that unsaturated fatty acids would be destroyed by BF3 due to the low concentration we used (see response to previous comment on this).

Line 132: The actual figure 4 should become figure 2 and, consequently, actual figures 2 and 3 should become figures 3 and 4, respectively.

We will change the order of these figures in our final submission.

Actual figure 4: The upward displacement of either one or two GC trace(s) within each group would make the different trends easier to compare. The horizontal axis could also be homogenized with that of figures 5 and 6 (age or eventually both depth and age, and from right to left).

The overlap of the FAME concentration plots shows the strong coherence between the datasets, which wouldn't be as clear if they were offset, thus we prefer to keep it this way. We choose to plot this data against depth in this figure since this section is dealing with how the FAME concentrations change downcore and how the different compounds compare to each other and age is not particularly relevant until later in the discussion. It is not really possible to have both age and depth on the x-axis since the age model is not completely linear. We will provide the data in the supplement so readers will be able to look at both depth and age if they wish.

Line 144 and all along the manuscript: Please also give an estimated time span when speaking in cm depth.

We will add an estimated time span in our final submission.

Lines 166-168: In Dalsgaard et al., the mean proportion of C18:0 FA in Prymnesiophyceae is only 3%! Please specify it.

This is correct. We pointed out in lines 168-170 that, in this study, the majority of FAs produced were the unsaturated form which are preferentially broken down in the water column and sediments (Haddad et al., 1992; Matsuda, 1978; Colombo et al., 1997). Thus, although the C18 FA represents only 3% of the *total* FA fraction, its higher preservation rate compared to unsaturated fatty acids, increases its proportion in the sediment.

Line 170: 'higher preservation rate' may be misleading; replace with 'higher potential of preservation'.
We will change the wording accordingly in our final submission.

Line 170: replace 'its proportion' with 'its relative proportion'.
We will change the wording accordingly in our final submission.

Lines 166-181: This whole section deals with proportions of C18 FA in laboratory cultures which can show great differences with the environment. Could authors comment on this?

Since there are very few studies looking into the different algal producers of C18 FA it is tentative to comment on potential differences between the results of in vitro and in situ studies. In the current knowledge stage, laboratory experiments are essential to document which algae, and under which conditions, synthesize the C18 FA. We will add few words in our final submission on the fact that this finding is based on laboratory cultures and on the potential limitations.

Lines 181-183 and more generally: This is one of my main concerns. The C18 FA can be produced by various type of (micro)organisms and assigning a single origin to this compound is rather daring. Authors should definitely support their hypothesis and comment about other potential sources of this compound such as bacteria, macrofauna, zooplankton, atmospheric inputs, land plants… One would also expect concentration profiles to be combined with d13C values to strengthen interpretations on the origin of individual biomarkers.

See lines 308-316. Here we include the d13C values in our interpretation of the source of fatty acids which supports a pelagic phytoplankton source. Our suggestion of *Phaeocystis antarctica* as the main producer of C18 is clearly presented as the most likely dominant source based on the available information and is a conservative suggestion. We point out that contributions from other sources such as diatoms or dinoflagellates cannot be excluded. Inputs from land plants and atmospheric inputs are highly unlikely due to the location of the core (Antarctica) and the highly productive nature of the water column.

Line 184 and thereafter: The same comment (as that made for the C18 FA) holds for the C24 FA. In this case isotopic data could be additionally used to support a planktonic (vs terrestrial plants) origin.

We are very cautious in our interpretation of the C24 fatty acid and do not assign a specific source. As we point out, contributions from terrestrial plants are highly unlikely due to the lack of land plants proximal to the core and the highly productive nature of the water column in this area.

Line 200 and thereafter: This is true but the degradation rates of lipid biomarkers appear strongly dependent on the redox conditions. Could authors give information on the redox state at the water-sediment interface and the possible influence of bioturbation in the surficial sediments?

The preservation of annual to sub-annual laminae throughout the core indicates very reduced bioturbation and the presence of dysoxic to anoxic bottom waters. However, we argue that much of the degradation takes place within the water column which is well-mixed and oxygenated, as well as in the surface sediments. This is a highly productive environment involving many trophic levels thus recycling of material in the water column will be substantial resulting in anoxic bottom waters. We don't have information on the redox conditions, it has never been undertaken and this would be very difficult to monitor at such a remote and hostile location.

Lines 227-229: Could this be due to an impact of bioturbation and/or to microbial production within the sediment?

This is highly unlikely due to lack of bioturbation and anoxic bottom waters. While we cannot rule out anaerobic microbial production in the surface sediments, this appears to be unlikely due to the consistent profile of FA homologues. If there was a major contribution from in situ microbes, we would expect a change in the FA profile such as the presence of branched fatty acids etc. in younger samples.

Lines 257-259: A similarity between the concentration profiles of C18 FA, HBI triene and HBI diene is not obvious in figure 5. Authors are encouraged to reconsider/specify those words.

In lines 257-258 we state that "one key similarity between both the HBI diene and triene, and the FA concentrations is that the highest concentrations are found in the youngest sediments." Figure 5 is split into two sections – the 1550-1950 period and 1950-2000 which have different y-axes. The y-axes for the 1950-2000 period (shown on the right) have much higher values for all four plots than the older period (shown on the left) since the concentrations in this period are much higher. Plotting the whole record on the same y-axis would mean that the plot is dominated by the high concentration in the top part of the core and the smaller-scale changes would not be visible, hence choosing to split it up. Thus, the similarity between the fatty acids and HBIs in having higher concentrations in the top part of the core is clear from the higher values in the y-axes on the right-hand side of the figure. The higher concentrations of fatty acids in the top of core are clearly shown in Figure 4.

Lines 261-262: This sentence is not clear. Do authors mean: '.... and to diagenetic transformation within the sediments including sulfurisation (ref), isomerisation (ref) and cyclisation (ref) reactions'

We will amend the sentence in our final submission to: "Concentrations of HBIs are also susceptible to degradation through the water column through visible light induced photo-degradation (Belt and Müller, 2013) and diagenetic effects within the sediments including sulphurisation (Sinninghe Damsté et al., 2007), isomerisation and cyclisation (Belt et al., 2000)."

Lines 262-264: This statement is misleading and in contradiction with section 3.4. Clearly, one cannot speak about a better preservation in the top sediments. The concentrations of HBI reflect the flux of lipids reaching the seafloor while the decrease in concentration downcore reflects enhanced degradation in the first cm of sediments (yet possibly including variations in productivity).

When we say better preservation, we mean that younger sediments have been less affected by diagenetic effects since degradation increases over time, and that explains why we have higher concentrations in the top of the core. However, in our final submission we can change to the wording slightly to make it clearer. We agree with the reviewer that concentrations in the core will reflect both the flux of lipids reaching the seafloor and diagenetic effects. We discuss this in lines 265-273 where we suggest that changes in concentration will be affected by both changes in preservation processes and any change in production of compounds in the surface waters.

Lines 272-273: I agree but this holds true if diagenetic conditions remain the same through time. Any indication on potential variations in the redox state of the water column and water-sediment interface back in time?

Unfortunately, we do not have data on the redox state of the water column as it has never been undertaken. Mn is sometimes used as a proxy for redox conditions at the water-sediment interface (Jimenez-Espejo et al., 2019). Unfortunately, this element has not been measured in DTGC2011 core.

Line 290: Again the concept that preservation of organic matter is better in surficial (younger) sediments is unfounded and in contradiction with section 3.4. It should be revised throughout the whole manuscript.

As mentioned above, in our final submission we can change to the wording slightly to explain this more clearly.

Lines 311-314 and thereafter: I don't think such a difference can be considered really significant (keeping in mind that the reproducibility was +/- 0.26 per mill). This statement might be a little far-fetched and I would suggest to remove it.

We are not convinced that a difference of 0.5 per mil should be dismissed as insignificant, since it is twice the reproducibility level and this is based on the mean of all samples in the core. Besides, this is only a tentative suggestion and is certainly worth considering. We will present it as such in the revised version.

Lines 355-356. I am not convinced by this statement when looking at the d13C profile of the C24 FA which shows a clear trend towards lighter values (2-3 per mill) within the last 150 years. Authors are mentioning this trend later on (lines 375-376). Could this be linked to increased land plant inputs due to ice retreat?

Over the last 200 years, the C24 FA d13C values initially trend towards more positive values before becoming more negative in the last ~100 years. In contrast, the C18 FA shows no clear long-term trend over the past 200 years except for a relatively rapid shift towards more positive values after ~1950 C.E. If atmospheric CO2 was a key driver of fatty acid d13C, then we would expect both compounds to respond together, showing a trend towards more negative values over the last 200 years which neither of them do. However, we thank the reviewer for highlighting the trend in C24 FA and we will add an additional sentence to point this out.

We do not believe that the trend towards more negative values in the C24 FA d13C would be due to increased land plant inputs. It is true that parts of the Antarctica Peninsula have experienced an increase in mosses due to recent ice retreat, however, there is no evidence of recent ice retreat in Adélie Land and very few land plants are present in this area. Our core site is situated 50km off the

coast meaning that even if there were land plants in Adélie Land, their contribution would be very low.

Lines 366-367: please temper with 'do not tend to produce high proportions of this compound'.

We will amend the sentence as suggested.

Lines 372-378: This again is somewhat speculative. If both FA have distinct origins, than the diagenetic impact on their 13C composition may be significantly different. What about the possibility that either one or both FA are being produced in the top sediments?

We disagree that the C24 and C18 would have such different diagenetic pathways. While we cannot rule out this suggestion completely, we are not aware of any literature to suggest this would be the case. As mentioned before, there is no evidence of any bioturbation or metazoan benthic activity in the sediments, and the bottom waters are known to be anoxic meaning any fatty acid producers within the sediments would be very limited.

Section 4.3 (lines 522-530) and conclusions (lines 540-541): As considered for FA in actual figures 2 and 3, a correlation table would help in highlighting putative relationships between lipid biomarker (concentration or d13C) profiles and specific phytoplanktonic species.

While we agree that this could be helpful, unfortunately the biomarker and diatom data were taken from adjacent samples (thus have different depths and ages) meaning they cannot be directly compared (unless they were resampled which would introduce errors). In contrast, comparison of different fatty acid compounds was possible since they which were all present within each sample meaning they were analysed simultaneously. Furthermore, due to the nature of the data, having high frequency-high amplitude changes due to dynamic environment, and the fact that they are different types of data, we do not think that a correlation table comparing diatoms and biomarkers would be useful. For this reason, it is not common in palaeoclimatic to look at correlations and is generally considered more useful to look at broad coherence between datasets which may change downcore. Therefore, we think it is more useful to plot the downcore records together in order to see the coherence between them.