Reviewer 1

The manuscript "Whole water column distribution and carbon isotopic composition of bulk particulate organic carbon, cholesterol and brassicasterol from the Cape Basin to the northern Weddell Gyre in the Southern Ocean" by Cavagna et al presents particulate organic carbon (POC), cholesterol and brassicasterol concentrations and their stable carbon isotope composition. The aim of the study is to unravel particle fluxes in the Southern Ocean (Weddell Gyre) which is within the scope of BG. The concept is not new but applied for the first time to the study area. Based on their data, the authors conclude that zooplankton fecal aggregates play a key role in carbon export for their study area. They also hypothesize that the release of sea-ice algae influences the isotopic signature of sterols in the open ocean.

In my view the authors particularly present interesting depth-related changes of the ratio of brassicasterol vs. cholesterol. However, the major conclusions regarding the contribution of heterotrophs and autotrophs are not substantiated by respective biological data and, therefore, quite speculative. I also have the impression the analytical aspects of this work could be improved (see detailed comments below). For an improved representation of the data it would be helpful to present the analytical thresholds:

- What are the limits of detection and quantification for the biomarkers and POC?
- How much POC would you need to reliably evaluate the stable carbon isotope composition (see also comments below)?
- A reproducibility study based on n = 3 runs of the complete analytical procedure applied to one sample would improve the technical quality of the paper and would help the reader to assess the impact of the analytical variability.

I also think the manuscript would benefit from professional editing to improve language (detailed comments below). The title could be shortened (e.g. "Carbon isotopic composition of bulk particulate organic carbon, cholesterol and brassicasterol from the Cape Basin to the Northern Weddell Gyre in the Southern Ocean"). The conclusion chapter contains some discussion and summary.

REPLY: We would like to thank Reviewer 1 for his/her insightful comments & suggestions. The detection and quantification limits for LP-POC (large particles POC) are based on blank measurements of Ag filters: POC = 1.06 ± 0.06 µmol (detection limit = 3x0.06; quantification limit = 5x0.06). The detection and quantification limits for SP-POC (small particles POC) are based on blank measurements of QMA filters: POC = 1.43 ± 0.15 µmol (detection limit = 3x0.15; quantification limit = 5x0.15). The equivalent POC blank concentrations for a volume of 100L is 0.01 µM which lower than all the concentrations presented in Table 1. The total volume filtrated by the High Volume Filtration Systems ranges between 152L (surface water) and 1983L (deep water). POC concentrations are now presented with two decimals, Table 1. The detection and quantification limits for the biomarkers have been defined as the cutoff limit of 100mV peak amplitude (GC-c-IRMS analysis) to accept or reject results. The method section of the paper has been updated with this information.

Concerning the reproducibility: unfortunately, we can no longer run replicate filters from this dataset (n = 3 runs as suggested) since samples used for POC analysis are 25mm filters fully combusted during the Elemental Analyzer (EA) combustion phase. However, as detailed above, this has been done for blank measurement.

The title has been shortened and the revised manuscript has been proof-read by a native English speaker.

Font sizes and axis labels should be increased. Also, there is some redundancy between figures and tables (e.g. Table 1 and Figure 4). In my view, some relevant literature is not considered (e.g. Fisher, 1991, Marine Chemistry, 35, 581-596).
In summary, the manuscript still needs substantial revision in technical aspects, discussion and style.
REPLY: In accordance with Reviewer 1 comment, the manuscript has been substantially revised in its technical aspects, both Figures and Tables, and text. Concerning Table 1 and Fig. 4, we consider it important to provide raw data in the form of a Table such as visual depth variation (Fig. 4).
P1669 L16: "could be applied as proxies" -> done **L21** full stop missing -> done
P1670 L21 comma missing after O'Leary et al. (2001) -> done **L23** "All three studies"? -> done
P1673 L9/10 unclear, rewrite -> done **L14** delete "but" -> done **L15** "before being advected" -> done **L18** delete "occupation" -> done **L21** in Table 1 a maximum of only 11 samples are displayed -> corrected **L25** replace "in-situ large volume filtration systems" by HVFS -> done **L29** "prior to use" -> not sure what is meant with this comment
P1674 L3 replace "during" by "for" -> done **L10** I would suggest using "1-53µm" throughout the ms -> done **L21** replace "during" by "for" -> done **L17** important statement – any published reference? -> don't understand which statement (mistake in the line number?)
P1675 L9 "assume that the isotopic" -> done **L10** is there any way of measuring the residue? This would add another level of analytical quality
REPLY: Assuming that this comment refers to the isotopic signature, the answer is that to the best of our knowledge, nobody tried to measure the isotopic signal of the residue since the residue is highly difficult to recover at 100% itself. This means that uncertainties on residue isotopic value would stay (checked by Boschker et al. using an internal standard directly at this step).
L12 is this paragraph based on the method of Boschker as well? -> Reference -> done
P1676 L6 ", and (ii) gas chromatography-mass spectrometry (GC-MS)" **L14** replace "possible" by "potential" -> done **L22** "trimethylsilyl" throughout the ms and captions -> done
P1677 L1 is there a reference for this procedure? -> done
P1678 L1 what are the values for the detection limit? -> for this study, the cutoff limit considered adequate to accept or reject results was set at 100mV signal size intensity (GC-c-IRMS). This cutoff limit is based on analytical data showing no consistent variations in $\delta^{13}C$ signatures of internal standards within this range (see below). **L5** "for that purpose" -> done **L18** turbidity sensor attached to CTD? Any signs of a nepheloïd layer? -> There was no turbidity sensor attached to the CTD. However, as mentioned in the manuscript, one sign of nepheloïd layer is the observed increase of LP-POC over T-POC ratio apparent near the seafloor. **L22** "due to the analytical method"; the heterogeneity of the natural system is of course hard to assess. However, the analytical errors could be easily checked by complete triplicated runs of a given sample -> the analytical method is not at all responsible for the large standard error: this sentence has been modified **L25** "brassicasterol at depths below 750m was below the detection": what is the detection limit? This is also important for the validity of the biomarker ratios. -> see responses above
P1679 L17 "similar to S1" -> done **L23** "towards the seafloor" -> done
P1680 L18 "systematically and reached a value" -> done **L22** delete "very" -> done
P1681 L1 "limit in the deeper" -> done **L7** "converged" -> done **L8** what do you mean by "eastern route" -> removed **L12** Hedges et al. (2000) might be a good reference here as well (Organic Geochemistry 31 (2000) 945-958) ->

REPLY: We feel that this reference does not really fit here, see Wakeham et al. (2009) to support this point of view: "*Molecular level, hydrolysis/chromatography-based characterizations indicate preferential loss of labile organic compounds as OM sinks, leaving behind a residue enriched in refractory material and compounds added by heterotrophic decomposers (Wakeham et al., 1997a). In contrast, techniques that ''see'' both the chromatographically ''characterizable'' and ''uncharacterizable'' fractions of bulk OM (Hedges et al., 2000; Lee et al., 2004), such as solid state ^{13}C-nuclear magnetic resonance (Hedges et al., 2001) and direct temperature resolved mass spectrometry (Minor et al., 2003), show that the two fractions have similar compositions that might reflect preservation of OM by association with an inorganic matrix.*" .

L19 "Taking into account the time lag" -> done **L26** delete "rather" -> done **L29** delete "rather" -> done

P1682 L5 delete "since" -> done **L7** ". The discussion is focused on" -> done **L13** and **L15** "barium" lower case -> done **L23** I wouldn't term high variability a "trend" -> done. We renamed it as "observations"

P1683 L3 delete "as already highlighted in the Results section" and begin with "The general…" -> done **L6** "the decrease of bra:cho ratios reflects (…) its stabilization around 1 in the deep water" doesn't make sense – rewrite -> done **L8** "reaching a maximum between 500" -> done **L9** Of course bra:cho of 1 reflects similar concentrations of both compounds. However, as there is no systematic or kinetic relationship between both compounds I wouldn't call it "equilibrium" here -> done: equilibrium is replaced by stabilization **L21** replace "becomes" by "is" -> done **L25** this belongs to conclusions -> done

P1684 L13 "estimating an" -> done **L19** "throughout the entire water" -> done

P1685 Could you give epsilon values here as well for comparison?

REPLY: Fright and Wainright (1991) do not calculate epsilon values, they measure growth rate and they measured the isotopic signatures. That is how they discuss the growth rate related effect. Popp et al (1999) do base their calculation study on previous laboratory experiments in the case of sterols, we mean $\varepsilon_{sterols}$. For a maximum of transparency, we cite Popp et al. (1999): "Following results of recent laboratory experiments (Bidigare et al., 1997; Schouten et al., 1998), we adopt $\varepsilon_{sterols}$ = 7‰ and ε_{phytol} = 4‰". It does not make sense to provide epsilon values here since this is not calculated in the paper from Fright & Wainright and since the epsilon sterols is discussed before in the paper using the same reference. For more numbers details please refer to Popp et al. (1999).

Generally I would suggest combining the following very short chapters -> do not agree

P1686 L24 unify spelling of "fecal" in the ms -> done

P1687 L8 "increase of 1-2" -> done **L11** delete "then" -> done **L13** delete "they studied" -> done **L25** "somehow" sounds vague -> that is indeed the idea since no definitive conclusion can be proposed for the moment.

P1688 L3 "of the cell membrane" -> done. Do you mean "13C-12C" covalent bond here? "13C-13C" would be very rare -> we agree, this is however a hypothesis which deserves to be presented **L15** delete "all dataset"? -> better to let it "d13C SP-cholesterol = (0.7 x d13C SP-cholesterol)": one of these should be brassicasterol, correct? -> yes, corrected **L20** unify "CO2 (aq)" throughout the ms -> done **L25** "when focus"? -> done

P1689 L3 "in the cold" -> done **L14** "all dataset"? Delete? -> We prefer to leave this information in the manuscript "mainly control the isotopic" -> We don't really agree with this correction. The good correlation "reflects" and not "controls"

P1690 L2 are these epsilon values?

REPLY: no, these are the observed decrease of natural isotopic signature of (LP + SP) cholesterol & brassicasterol from the northern to the southern part of the transect; in accordance with the increase of surface CO2 aq. concentration **L18** "to the contribution of larger cells (diatoms)." -> done **L20** with only three slopes to compare your statement is quite weak…
REPLY: Yes - we are aware of this weakness. That is the reason why we mainly base our discussion and conclusion on hypotheses, highlighting the necessity to improve the database to formulate strong interpretation **L25** reference? -> done (Hedges et al., 2001)
P1698 How do you explain a 5.7 per mille change within 10m depth interval (S2)?
REPLY: We do not have a well supported explanation for such a variation over such a small depth interval. However, looking on the data (Table 1 – $\delta^{13}C_{LP\text{-}POC}$), it appears that the 2 close-by values of -25.1 and -19.4‰ at 2900 and 2910 m, respectively, are themselves enclosed by a value of -16.9‰ at 1450m (close to the value at 2910m) and a value of -26.0‰ at 3940m (close to the value at 2900). We have perhaps sampled the variability inherent to the deep ocean in this location? One point to highlight however: this does not affect the average estimation for deep ocean since these to values range with the one which are above and below.
Are the concentrations too low for correct isotope ratios? As demonstrated previously we are well above the detection and quantification limits for POC. If yes, you might have to eliminate other values as well. 0.0 µg L-1 would imply that there are also no stable carbon isotopes. -> this has been changed in the revised version by showing values with two decimals, as explained above. I didn't quite understand why the values in this table are different from previous tables. -> we do not understand this comment.
P1699 Caption: there is no "ND" in the Table -> yes there is, in the $\delta^{13}C$-cholesterol data column. However, Table 1, 2 and 3 and now summarized in Table 1.

Reviewer 2

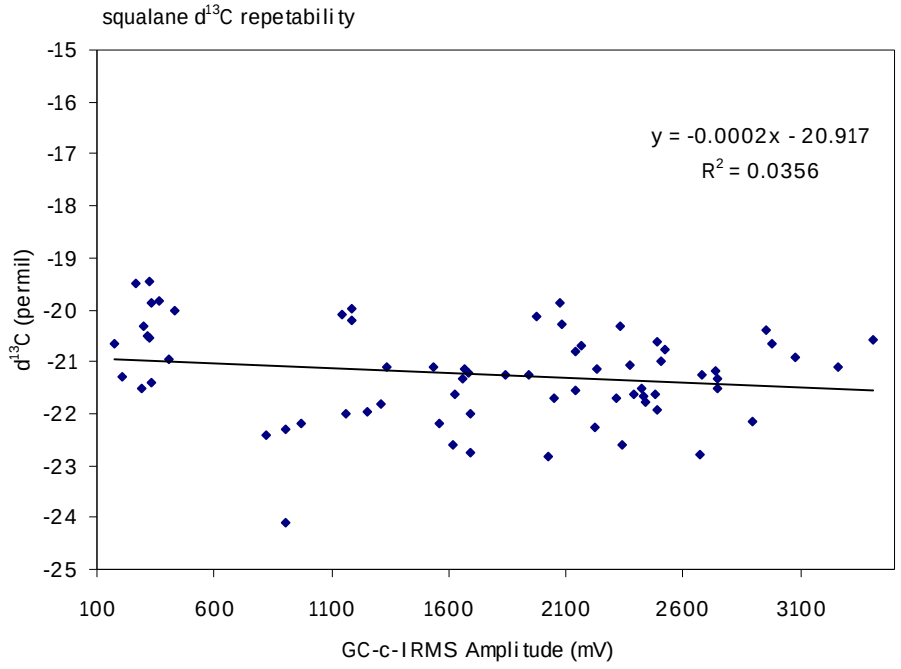
The authors investigate the concentrations and carbon isotopic composition of the bulk particulate organic carbon, and two sterols (cholesterol and brassicasterol) in the whole water column from the Cape Basin to the northern Weddell Gyre in the Southern Ocean. The manuscript is suitable for publication in Biogeosciences because there is a great need to understand the factors that determine C isotopic signatures of autotrophic and heterotrophic organisms, as well as their changes through the water column. The potential use of the carbon isotopic ratios of marine biomarkers as recorders of CO2 levels has also been reconsidered, and, although previous works already proved the relationship between surface CO2 concentrations and d13C of lipid biomarkers from the surface waters, here they show that the relationship might also be valid in deeper waters. It is regrettably that a study designed to look at the isotopic composition of biomarkers simply did not calculate the photosynthetic carbon fractionation between the inorganic carbon source and that of organic carbon synthesized by autotrophic organisms (epsilon p). Potentially clearer relationships between epsilon (p) and CO2 concentrations, as well as other environmental conditions, e.g. nutrients, could provide more insights on the factors that affect the carbon isotopic fractionation in the study area. Although the discussion and conclusions are not exciting because mostly based on hypothesis, the study contributes to a better understanding on the fate of organic material exported to the deep Southern Ocean.
REPLY: We would like to thank Reviewer 2 for his/her insightful comments & suggestions. It is indeed unfortunate in hindsight that no samples for $\delta^{13}C$-DIC were collected, and as far as we have been able to verify, none of the co-workers on this cruise have samples remaining which

would be adequate to perform these analyses at this stage, thus prohibiting us to calculate $\delta^{13}C$-CO_2 aq. from DIC speciation and $\delta^{13}C$-DIC. While we have considered using literature datasets to estimate these proxies, we feel this approach is likely to be flawed due to numerous uncertainties. Therefore, we do not develop this aspect in the present manuscript, and focus on the variation in $\delta^{13}C$-POC and $\delta^{13}C$-sterols in the water column.
On the other hand, our focus on $\delta^{13}C$-POC and $\delta^{13}C$-sterol variations along depth profiles and thus including the aphotic zone (meso- & bathypelagic zones) also represents the originality of our dataset compared to the available literature. We consider this manuscript to offer a first insight of on the fate of organic matter based on relatively high-resolution CSIA data. This approach allows a greater resolution and different timeframe of observations than samples obtained from sediment traps. Indeed, we hope that this study will lead to the acquisition of further similar datasets (including $\delta^{13}C$-DIC and $\delta^{13}C$-CO_2 aq.!) and an improvement of our understanding of organic matter flows in the open ocean.
According to the scientific and technical aspects, the work might be acceptable for publication in this journal, after a significant revision.
Some other criticism and comments are given below:
Table 1 and 2 could be merged into one table, to facilitate the comparison of d13C values for POC (suspended particles) to those of sterols.
REPLY: we agree with this suggestion and have merged both tables
P1673 L25 the water volumes sampled with large volume filtration systems should be specified.
REPLY: we have specified filtration volumes in the revised version
Table 1 the number of replicates used to calculate the standard error on d13C measurements (SD = +/- 0.10 ‰) is missing.
REPLY: This is based on 15 measurements – this information has been added in the revised version Table 1 legend.
P1675 L5-6 it is unfortunate that the authors did not use a surrogate standard to account for losses, such as, 5α-androsterol, to take into account the losses? Was the data corrected for these underestimations?
REPLY: We opted to present data that were not corrected for such underestimations, and to mention the degree to which concentrations could potentially be underestimated. This approach does not influence the observed sterol concentration ratios and the patterns we find in these ratios, if we assume that the degree of underestimation is similar for various sterols. This is now explained in the revised version.
P1677 L15 states that uncertainty of d13C was calculated by propagating standard deviations from triplicate measurements and correction for derivatization. What was the s.d. for the derivatization?
REPLY: We're not entirely sure that we understand this question correctly. We hope to answer it by sharing the measurements for the 5 standards sterols used to evaluate correction for derivatization.

GC-c-IRMS	non-sylilated sterols		sylilated sterols	
	$\delta^{13}C$ (average, n=3)	stdev	$\delta^{13}C$ (average, n=3)	stdev
cholestane	-27.76	0.26	-28.09	0.12
cholesterin	-24.47	0.60	-26.62	0.48
campesterol	-29.09	0.86	-30.92	0.15
stigmasterol	-30.45	0.07	-31.69	0.13
b-sitosterol	-29.77	1.04	-31.26	0.27

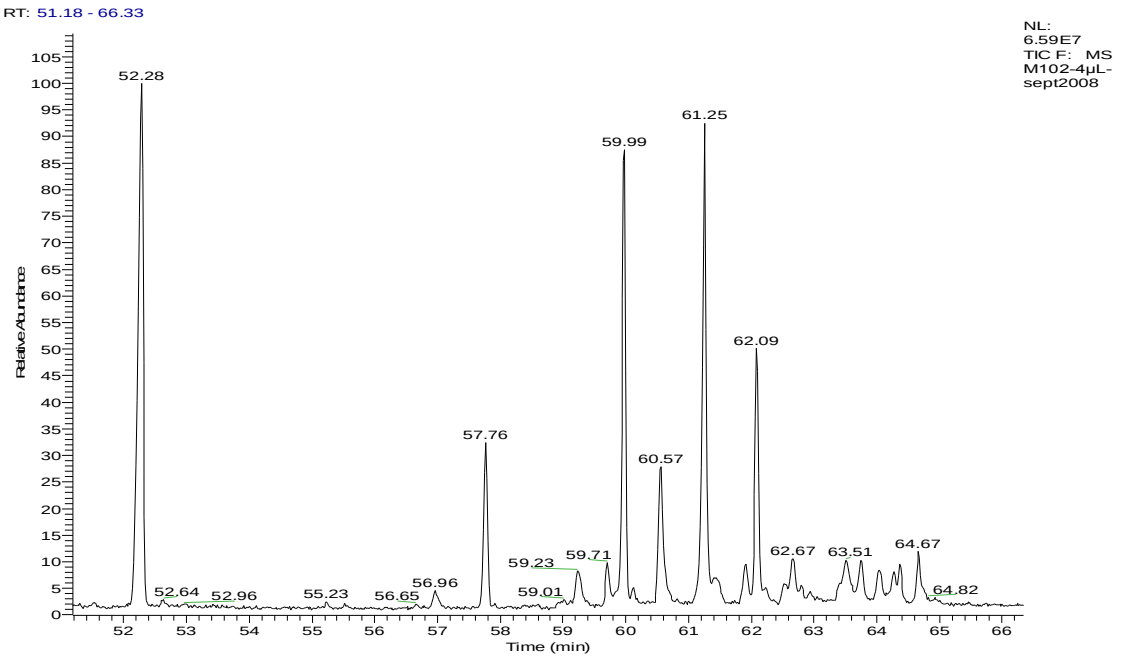
I have some concerns on the compound specific isotope analysis: the accuracy of the d13C measurements are impacted by signal size or linearity of the GC-c-IRMS (Sherwood-Lollar, B. et al., 2007, Analytical Chemistry 79, 3469-3475). Were these parameters taken into account for the whole range of concentrations, noting that in deep waters the concentration levels were very low and could be the reason of the enrichment of d13C-cholesterol with depth? Was the signal size for the sterols higher than 0.5V? Besides abundance, the isotopic data of sterols might be difficult to obtain due to co-elution of other sterols. Was the chromatographic column (DB-5, 30m x 0.32 x 0.25 um) good enough to resolve the target sterols from their saturated counterparts (cholestanol & C28_22)? I would be useful to provide some comments on this or a chromatogram in the supplementary material showing how the sterols are separated. Equation (1) seems to be incorrect since the tri-methylsilyl group (TMS from the BSTFA) contains 3 carbon atoms. Therefore the corrected formula should subtract 3 x the 13C of BSTFA, and n should be the number of carbon atoms of the sterol without derivatization, and not the number of replicates!!!

REPLY: Indeed, this was obviously a typo mistake in the original version, this has been corrected. As already mentioned in our response to Reviewer 1, the cutoff limit we considered adequate to accept or reject results was set as 100 mV. This is significantly lower than the signal size suggested by Reviewer 2. We are aware that this aspect can be subject to debate, but several aspects need to be kept in mind. First, from the data presented in Sherwood-Lollar et al. (2007) we principally retain that variability increases at low concentration. Secondly, although they suggest an enrichment of the $\delta^{13}C$ signal at low concentrations may occur, this conclusion is not as convincing and is more likely to vary depending on the specific IRMS system and tuning, and on the type of compounds and chromatography considered. The degree to which signal size influences stable isotope data is well known to be highly dependent on instrument settings, and from our data we have no reason to assume that our values would be substantially biased at lower peak amplitudes but above 100 mV. Indeed we provide below a graph showing the correlation of $\delta^{13}C$-squalane vs. peak amplitude (n = 69 measurements): as you can observe, no significant trend is observed, supporting our confidence on data validity.



We are also convinced that the chromatographic column was good enough to resolve the target sterols from their saturated counterpart. Below we provide an example of the GC-MS chromatogram, zoom in to show sterols peaks. Another point to be highlighted is that in deeper waters, there is a lower diversity of sterols, hence chromatograms of deep water samples show a lower number of peaks compared to surface samples, resulting in a lower risk of coelution.
Example: Station 5 - 50 m depth – zoom on the sterols

Retention time = 52.28 min => squalane / internal standard
Retention time = 60.57 min => cholesterol
Retention time = 61.25 min => brassicasterol

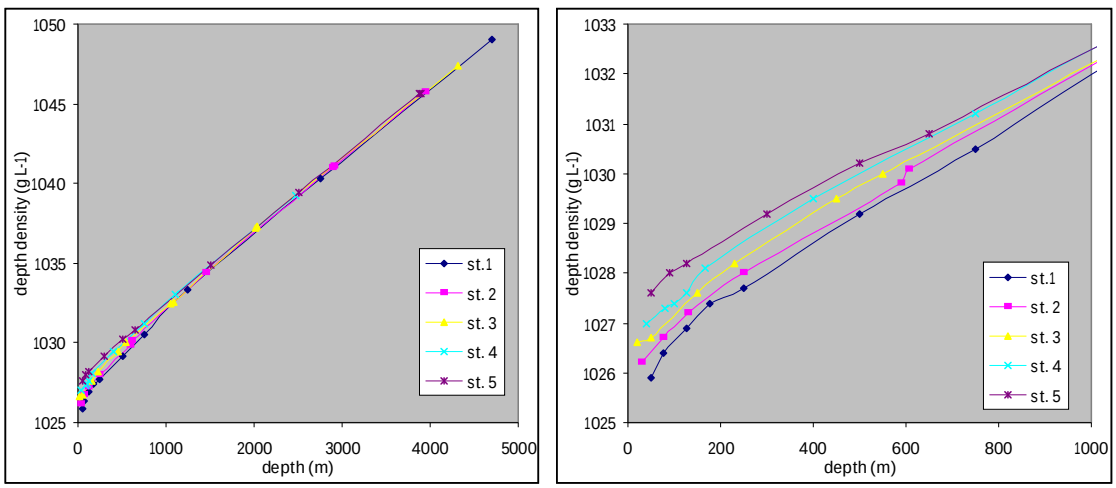


Once Retention time for cholesterol and brassicasterol is well defined, we use exactly same column and same program for GC-c-IRMS and work on raw data using retention time to find back compounds we are interested in (squalane, cholesterol, brassicasterol). If asked, we would be glad to share an example of raw data excel file to show how we have worked.
Figure 2. I consider that this figure is not necessary for the content of the paper. Otherwise, as this figure is compiled of 2 graphs, I would recommend adding letters to the different graphs included in each figure to be referred with the text. I recommend starting from the left to the right (A for the left figure and B for the right figure).

REPLY: We prefer to keep this figure in the manuscript since it clearly shows our approach for data corrections. This is however updated following the suggestions of reviewer 2. Regarding the suggestion of adding a chromatogram in an annex, we would have followed it if the aim of the paper was description of chromatogram and observation of sterols series. This is not the case: this work is focused on two well-known sterol biomarkers and their variation in a studied natural system. We hope the chromatogram shared for responses to reviewers bring the requested information.

P1678 L1 What are the detection limits fro brassicasterol? They should be specified in Table 2 and 3. -> done **Figure 3**: Since you do not discuss the data based on the water masses distribution. I would recommend for clarity, to plot the parameters vs. depth rather than density or better show 2 y-scales.

REPLY: This way of showing depth profiles allows type of zoom in the surface & mesopelagic water (we would say that we are in between the classic depth meter scale and the logarithmic scale for the first 1000m in the water column). In fact, correlation between depth density & depth meter is linear below 1000 m but in the upper 1000 m this correlation is not linear (see figures below) => work with depth profiles in term of depth density allows working with a better visibility of surface and mesopelagic depth variation of the studied parameter (here POC and sterols in larges and small particles). We also think it is more accurate to present depth profiles vs. depth density than depth profiles vs. depth meter; and this observation is not only valuable for water mass study. In other words, for the sake of accuracy we prefer to show our profiles using the density, but propose a double axis for Fig. 4 to demonstrate the relationship between depth and density. We did not add the double axis on Fig. 3 so far since it makes the figure somewhat overloaded. If the editors and/or reviewer(s) prefer, we can obviously change this.



P1680 L22 Looking at the Table 3, large particles in surface waters have not slightly larger brassicasterol/cholesterol ratios than underlying waters, but just station 5!!!

REPLY: Right, this has been corrected in the revised version.

P1681 L27 "At S4 within the upper 100m d13C LP-POC and d13C SP-brassicasterol decreased with depth". Due to the plot with density and not depth it is difficult to see this statement from Fig. 4. As I said before, I recommend changing the density y-axis for the depth-axis.

REPLY: Please refer also to Table 1. This is not easy to visualize mainly because this observation concerns 2 to 3 depth values in the upper 100 m and not because we are showing the dataset vs. depth density rather than depth meter. Indeed it would be harder to see this statement if we were showing data vs. depth. We hope that our explanation above is enough to convince Reviewer 2.

4.1. Section: Variation of brassicasterol and cholesterol content with depth. The outcomes of this section are not novel, and the data presented are for the most part sound. I suggest presenting Figure 5B separated from Figure 5A, since Figure 5B is discussed under another section. Also, the Figure 6B (zoom of the panel A) could be removed and Figure 6B integrated together with Figure 5A.

REPLY: Since we have the possibility to share graphs which helped us to interpret our dataset we prefer to do so. We feel that Fig 5 A and Fig. 5 B can be shown together as parts of the discussion. Fig. 6 has been removed in the revised version though the slopes obtained from SP-cholesterol vs. SP brassicasterol concentration in the water column are discussed, but we understand that showing correlations graphically does necessarily add important information.

4.2. Section: The authors stated that the d13C values of -7‰ obtained below 500m Is in accordance with previous laboratory experiments. However, they cite Bidigare et al., 1997, which only provides values for alkenones and not sterols. As the estimated differences between the d13C content of the biomarker and the biomass vary from -2 to 8.5‰ for different cultures of phytoplankton taxa (Schouten et al., 1998; Riebesell et al., 2000 and summarized in Hayes, 2001), it is recommended that the authors integrate this data variability in their discussion and not just take the value -7‰.

REPLY: Here, we do not look at differences between $\delta^{13}C$ of various biomarker and bulk biomass, but look especially at this relationship for sterols. This relationship has been calculated as being -7‰ (Schouten et al. 1998; cited precisely like that in Popp et al. 1999). In Riebesell et al. (2000) the spread is proposed for different classes of lipids on not only for sterols. However, in sake of transparency, we cite the mean isotopic difference between POC and sterol -8.5 ± 1.1‰ obtained by Riebesell et al (2000) and the range cited by Hayes (2001): "It appears common, but far from universal, for MVA-pathway sterols to be depleted relative to biomass by 5-8‰ (and not 2 to 8.5‰ including the phytol). Bidigare et al. 1997 reference has been removed.

P1684 L21 The authors hypothesize that the stable value offset of -7‰ below 500m indicates that brassicasterol in synthesized in the surface water and not below. However, this hypothesis is a bit flaw since the components that integrate the bulk POC degraded at different rates, and the offset between d13C primary photosynthate and d13C eukaryotic biomarkers does not necessarily reflect the offset between d13C-POC and d13C-biomarkers in the waters below the euphotic layer. In other words, the assumption that the d13C of POC is assumed to be that of the phytoplankton might still be valid in the euphotic layer but it can become less reliable in the deep layers, where phytoplankton is not necessarily the major component of the POC.

REPLY: the authors assume that this hypothesis is valuable only for the small particles and because in this case they observed $\delta^{13}C_{SP\text{-}POC}$ is stable within the entire water column. Thus, because $\delta^{13}C_{SP\text{-}POC}$ is stable within the entire water column, it allows checking variability of $\delta^{13}C_{brassicasterol}$ with depth. Below the surface water the offset is stable indicating mainly that no change occurs on isotopic signal of brassicasterol => it is synthesized only in the surface water.

P 1684 L24-28 if the Suess effect should be responsible for the enrichment of d13C cholesterol with depth, the same enrichment will apply to the d13C of brassicasterol and d13C-POC.

REPLY: if the Suess effect should be responsible for a lighter signal penetrating from the surface to the deeper water letting the deep ocean signal heavier because not concerned yet by the Suess effect, we should observe the same trend for brassicasterol than for cholesterol: we agree with Reviewer 2. Therefore we choose to remove this paragraph since it does not lead additional information and Suess effect does clearly not have to be considered to explain variation observed via our dataset.

Another factor that affects the carbon isotopic fractionation of the algae is the change in irradiance and this should also be discussed in section 4.2.

REPLY: this is now integrated in the revised version of the manuscript (section 4.2.1.)

P 1685 L15-20 if the enriched cholesterol in the deeper waters comes from a previous bloom from the surface waters, enrichment for brassicasterol should also be observed. Moreover, higher concentrations of POC and sterols will likely be present in the deeper waters.

REPLY: we agree with Reviewer 2, a $\delta^{13}C$ enrichment due to growth rate related effect should be visible also for brassicasterol. Unfortunately the signal for brassicasterol was not detectable in the deeper water for station 1 and 2 limiting our interpretation concerning this compound. The manuscript is built as offering a list of various factors possibly acting on the observed variations; our actual dataset does not allow rejecting one or either of these factors and this way of presenting the study allows forming his/her own conclusion/interpretation. This is now highlighted in the revised version.

We follow variations of isotopic signals in parallel with concentration data since this combination allows observations which are not visible when looking only at concentration data. This is even more applicable when looking on the deep water particulate organic component composed by refractory material. The crossed isotope/concentration information provides better insight observation.

4.2.3. The hypothesis of the sea-ice algae related effect could be confirmed by the identification of specific sea-ice diatom biomarkers in the same samples, e.g. the IP25 (Belt et al., 2007 – Org. Geochem. 38:16).

REPLY: this is a very interesting suggestion and taken into account for further dataset acquisition. This would need the implementation of collaboration with scientific team working on this specific sea-ice biomarker. Because samples were collected during the KEOPS 2 expedition to implement the BONUS-GoodHope dataset for the Southern Ocean, and because several samples were taken for this purpose during SIPEX 2 expedition, we will do our best to test this hypothesis (collaboration with LOCEAN G. Massé team – Paris, France?).

P 1688 L1-5 the hypothesis on the high pressure on cholesterol biosynthesis occurring below the surface water seems very unlikely because kinetic isotope effects should have a minor contribution compared to the factors that affect the natural variability in carbon isotope fractionation among algal taxa: growth rate, T, dissolved CO2, cell geometry, irradiance, etc.

REPLY: the listed factors are acting on the surface water. We propose this hypothesis for the bathypelagic zone (below 1000m) where these factors do not act anymore on synthesis of compounds.

Why are the slopes shown in **Table 5** differing from those exhibited in Figure 7? If they are the same, I recommend integrating the information of Table 5 into the Figure 7 and removing Table 5.

REPLY: Apologize for this mismatch; the initial graphs contained a few errors. This has been corrected in the revised version. Information in by Table 5 complements Figure 7. We still feel

it's important to provide all information: providing the intercept, slope, slope standard error, *p*-value, R^2 is useful for the validity of observed trends. Moreover, adding all this information to Figure 7 would make it very complex, so we prefer to keep Table 5 in parallel with Fig. 7.

Reviewer 3

Cavagna and coauthors presented an interesting dataset of concentration distributions and carbon isotopic compositions of 2 biomarkers (cholesterol and brassicasterol) from a transect from Cape Basin to the Weddell Gyre. Different depths in the modern water are investigated. The manuscript is based on a large dataset, but in the present form on the ms the main working hypothesis and conclusions of the study do not appear clearly. The introduction needs to be reorganized. The discussion parts contain a large umber of titles and sub-titles that make the reading difficult. Similarly the manuscript contains a large number of figures (7!) and Tables (5!). Most of them should be clarified and the authors could think of removing some of them while reworking and reformatting the discussion.
The dataset is f interest for the climatic/paleoclimatic community and the manuscript will be suitable for publication in Biogeosciences Discuss. The manuscript should however be deeply reorganized and clarified. Language also needs to be proof read by a native English speaker. Specific comments are following.
REPLY:
The number of Tables has been reduced to avoid redundancy.
For the Discussion, we understand the request from Reviewer 3 is more a question of form (subjective) than a question of content (objective). We still feel it's important to subtitle the various hypotheses to assist the readers to follow the line of argumentation followed. The revised manuscript has been substantially edited and proof-read by a Native English speaker
P1667 title. The title is too long and should announce the main focus of the paper
REPLY: It has been changed in accordance with request from the three reviewers
P1669 L22 "The release of"…? Production of? ->
REPLY: No, we speak on the release of sea-ice algae at the time of the sea-ice demise (end of ice season in de Sea Ice Zone SIZ) – this has been highlighted in the manuscript
L23 to the end of the abstract: it should state the broader scope of the study (pCO2 reconstruction? Understanding of the ecological turn-over?), then how this study compare with previous studies and literature (in the SO and elsewhere in the world). Finally, before o explains the work plan, the working hypotheses need to be stated.
REPLY: First of all, as already mentioned in the manuscript, to the best of our knowledge there is no other dataset of small particles (suspended particles sampled via HVFS) $\delta^{13}C$ cholesterol & brassicasterol throughout the entire water column in the S.O. or elsewhere in the global ocean. For the $\delta^{13}C$-POC this is possible to find some depth profiles not listed in our manuscript because not focused on the system we are studying (e.g. Goni et al., 1997 – Nature) but they show a very weak resolution and do not attempt the seafloor but mainly mesopelagic layer. Secondly, as mentioned in our introduction, our first aim was to gain information on the fate of organic matter below the euphotic layer using sterols and their $\delta^{13}C$ (I would preferentially speak on fate of organic matter than on ecological turn-over since we are looking on molecules which show relative refractory nature allowing us to follow them in the entire water column); our observations led us to also discuss the paleo-pCO_2 reconstruction thematic whereas it was not our first aim. The submitted manuscript is a synthesis of the observations & interpretations made possible by the presented dataset. It is not a straight-forward scientific question/response paper and does not pretend to be such a

finalized work. We are however convinced (as the 3 reviewers, as we understand) that this dataset deserves to be published and available for the scientific community, already at this step even if it is not yet "exciting" in term of scientific response.

L2 set -> don't understand **L2-17** the first paragraph is not precise enough and sentences are too long.

REPLY: The manuscript has been proof-read by a native English speaker and reviewers 1 & 2 do not mention comment for introduction encouraging us to keep it in this form which is, we think, clear and easily readable.

P1670 L16-17 the link between the first and the second paragraph of the introduction is not clear and the transcription is abrupt.

REPLY: The link which has to be observed is: first paragraph (i) the scientific demand to improve our knowledge on what is happening below the euphotic layer, (ii) one way to improve our knowledge is to determine chemical composition of particles; second paragraph (iii) several studies have already examined $\delta^{13}C$ of POC in the SO but few (3) have examined $\delta^{13}C$ of specific compounds (sterols) and they were limited on the euphotic layer => we will present / we are happy to share with the scientific community first complete depth profiles in this study

L27 and elsewhere in the world? Are there other studies? Has the GEOTRACE program gathered and investigated such a dataset?

REPLY: there is no dataset like the one presented in this paper available in the world actually. As already highlighted in the paper, several studies have already proposed such dataset for $\delta^{13}C$-POC obtained from large volume filtration system but only for the surface water to close subsurface water. We focus on the Southern Ocean area and to the best of our knowledge, only 4 other publications present water column $\delta^{13}C$-POC in other region that SO (Goni et al., 1997, Nature; Nakatsuka et al., 1997, DSR I => data from sediment trap; Ostrom et al., 1997, GCA => data only for the upper 200m; Woodworth et al., 2004, DSR I => data from sediment trap) which do not lead to additional information compared to the publications restricted to the Southern Ocean already cited in the manuscript. Concerning sterols dataset, to the best of our knowledge, no other publication than the ones cited in the manuscript are available for such a dataset when looking at the surface water. No data are available at all for the complete water column in the global ocean.

This work is an original study which has been made possible through an expedition supported by GEOTRACE; however this program has not gathered & investigated such a database yet. This has to be (i) published and (ii) improved (in term of data quantity/quality – publications) before this possible further step.

P1671 L9 "a general biomarker": precise => please refer to the previous sentence in the manuscript **L9-15** what is the range of d13C cholesterol in individual organisms and between species? Is the range of variation large? If this is the case, could d13C variations reflect structure of the community?

REPLY: We are not able to answer in term of numbers since cholesterol is found in so many species from plankton to humans and this is not the scope of the presented work. However, especially focusing on the study of the manuscript we assume that the $\delta^{13}C$-cholesterol variations can not reflect structure of the community. Moreover this assumption is supported by Schouten et al. (1998) applying the term of general biomarker for cholesterol in the plankton community (eukaryotic biomarker).

P1673 L1-17 are these pieces of information needed for the discussion? If not, they could be deleted.

REPLY: this information allows understanding physical parameters of the studied transect, it is interesting to relate them to the fact that though the high dynamic of this area we conclude that δ^{13}C-sterols depth variation is operating in 1-D (strong surface to deep links) instead of 3-D (strong mixing & homogenization in the deep ocean) configuration (introduction L13-15 & conclusion)

P1674 L10 English: home-laboratory? => yes. This term is currently used in English **L25-25** the last sentence corresponds to results and no method presentation => agree, it has been removed

P1675 L6 case of reference => don't understand **L8** delete "max" => done **L22** "since the added IS amount is known": delete => done

P1677 L19 does contents stands for concentration? => Yes **L19 and below** this part needs to be rewritten => request not clear. This part is a description of variation observable from our dataset **L24** "similar magnitude": values are missing => this is not talking about values but more about magnitude. The magnitude is $10E^{+1}$, this is now précised in the revised ms.

P1678 L1 what is the detection limit? See response above for Reviewer 1 **L3** "variability due to analytical method": precise => don't find the sentence related to this comment **L6** the reason to provide density depth rather than regular depth is not clearly stated

REPLY: It is now highlighted in the new version. Moreover we propose here Fig. 4 with double axis though we are not convinced it brings additional information compared to the highlight of 100m and 1000m depth horizons as it was shown before.

P1681 L6-21 this part concerns the interpretations rather than the results

REPLY: we agree with reviewer 3, however it does make sense to treat this information directly in the result part since it details results observation for S1 and S2 mainly and since discussion on δ^{13}C-POC and sterols depth variation is more general. We hope Reviewer 3 will understand our point of view.

P1682 L5-9 how does this compare with core-top sediment?

REPLY: Unfortunately, sediments were sampled only at station 1 during this expedition. It is thus not possible to compare the dataset presented in the manuscript with related core-top sediment

P1685 what is the estimation of the time lag?

REPLY: Season to annual time lag (not decennia). However this paragraph has been removed in the revised manuscript (see Responses to Reviewer 2)

P1688 L9 in this entire part, it is difficult to see what the main message of the manuscript is and how its results compare with the existing literature. The authors should state at the beginning of this part, what their working hypotheses are based, on the existing literature. They should then discuss what new insight their results provide. => This is now highlighted in the revised version

P1689 L17-21 and P1690 L7-11 this belongs to the Results part

REPLY: the authors do not consider this belongs to the Results part because these are numbers obtained from Fig 7 & Table 3 which show data in a discussed form. Moreover these figures do not belong to the Results part but clearly to the Discussion part.

Figure 1: on the left hand side, the titles of the axis are missing (SST, latitude). The black bars on the SST color scale should be removed. On the right hand side, the vertical scale is not easy to compare with a "usual" depth scale (place a double axis). Again titles for the axis are missing (T, S). Since the salinity distribution is not discussed, could not the panel be removed?

REPLY: all information not found on the figure is in the legend mainly because we don't want to overcharge the figure. On the right hand side, this is a usual depth scale – this is now

clarified. Titles for the axis are T1 and S1 and are explained in the legend. We would like to keep visible salinity transect since most of the time temperature and salinity are shown together.

Figure 3 & 4: the labels and titles of all the axes are too small. On the vertical axis having a double scale with conventional depth would help.

REPLY: Indeed, figures are too small. This will be checked with the editing office. For the double axis proposition, see response to reviewer 2.

Figure 5: could you provide reader with the error bars on epsilon (either graphically or in the legend?) => Error bars on epsilon are now available on the legend