We thank the anonymous referee for the time they have taken to carefully and thoroughly review our manuscript. Their comments have helped to clarify our thoughts and have assisted us to improve the manuscript.

Our specific responses to each of the referee's comments are detailed below. The referee's original comments have been copied below, and our responses inserted in bold text. Page and line numbers refer to the original manuscript.

General Comments

This manuscript presents an assessment of the fate of microphytobenthos (MPB) production in a subtropical intertidal sediment. In this thorough study looking at yet another type of benthic environment and at yet another latitude, the authors deliver yet another set of data on the relative fractions and contributions of the different auto- and heterotrophic compartments of a sandy sediment. The data set is novel and advances our understanding of MPB processing. There have been relatively few stable isotope labelling papers looking at MPB, these have not always been in the field, and no other intertidal studies have been done in subtropical environments. Our study is also unique in that it directly quantifies more potential carbon loss pathways than any previous study of MPB processing, and was done over an extended time period (>30 d, compared to 4-6 d in previous studies).

With respect to the data set produced, this study is interesting and legitimate. However, with respect to the take-home message the authors try to deliver, the study fails. This work offers no evidence that a significant MPB biomass could be stored in the sediment to be qualified as "blue carbon". The novel idea presented by this study is interesting (MPB carbon storage), the topic is "sexy" (Blue Carbon) and I understand the rationale behind the work, but trying to derive some carbon storage potential from this study would be hasty.

Our study clearly demonstrates long-term (defined in our manuscript as >30 days) storage of carbon, which may ultimately result in "blue carbon" derived from MPB production. We believe this is clearly explained in the abstract and body of our manuscript. However, given that "blue carbon" is a current topic of interest, we acknowledge that the current title of the manuscript may lead the audience to expect that we have data showing longer-term storage of MPB production as "blue carbon". We have therefore altered the title of the manuscript to read "...*Potential for* long-term carbon retention...".

The potential significance of MPB storage suggested by this work is weak: 1) 50% of the production is lost (likely by resuspension) and 30% remains in the sediment. The rest is assimilated and respired. This is nothing out of the ordinary for this system and probably others (next point).

We see this as significant, with 30% of the carbon fixed within one tidal cycle remaining after 30 days (over which time there would be ~60 tidal cycles). Over time this equates to greater input than output of carbon fixed by MPB within the sediment. We have seen this in other instances also (e.g. Oakes et al. 2012) and view this as evidence that the carbon storage potential of MPB warrants further investigation. Given the high productivity of MPB and their ubiquitous distribution within photic sediments, which cover >30% of the world's continental shelf area (Gattuso et al. 2006), the global significance of longer-term storage of MPB carbon could be significant. We have added this information to the concluding paragraph of the manuscript.

2) This is expected as the experiment was done after a flood event and one more flood event happened during the study. As noted by the authors on p. 19794, "episodic freshwater flows are typical of any (sub) tropical system". Unfortunately the authors use a contradictory statement to support the potential for C storage in the following sentence of the same paragraph, and rather boldly in the conclusive section p. 19797: "there could be a substantial contribution of MPB to long-term carbon sequestration in unvegetated sediments. This is particularly remarkable given that the burial and retention of MPB-derived carbon in the current study was observed under conditions of high flow, when resuspension would be en-hanced."

We do not agree that this statement is contradictory. If loss of MPB carbon to resuspension is enhanced by high sheer stress during increased flows, then clearly there is less MPB carbon remaining to be buried and stored. The referee may be alluding to the lack of macrofauna at the site (see this referee's next point, below), resulting in reduced grazing and respiration of MPB carbon. However, given that episodic freshwater flows are typical of subtropical systems, any effect on macrofauna (low biomass, and therefore low grazing) would also be typical of this system. Furthermore, low macrofauna biomass is typical of intertidal tropical systems in general (Purwoko and Wolff 2008). We have added a short paragraph in the discussion to this effect (pg 19793, ln 24).

3) Macrofauna was absent and only foraminifera were sampled and analyzed, showing a negligible contribution to C processing. The absence of grazing is likely having a high impact on the MPB standing stock and recycling on the sediment organic matter. The absence of fauna is probably related to the highly dynamic characteristics of this benthic environment: high flow, resuspension of sediment and MPB, variation of salinity (1-24) will not allow macrofauna to thrive. Once again, should these episodic flood events stop, larvae would settle, biofilm and fauna would flourish and ultimately, the recycling of organic matter would be very different.

We agree that the lack of macrofauna at the site may reflect the highly dynamic environment and that the presence of fauna may alter organic matter recycling. The effect of low fauna biomass on carbon cycling has already been outlined in the manuscript on pg 19791 ln 21-22 (limited grazing may enhance retention), pg 19792 ln 24 (reduced burial/downward transport), pg 18793 ln 18-22, and pg 1974 ln 8. However, dynamic environmental conditions are typical of this and other (sub) tropical systems. Where fauna is more abundant we agree that MPB carbon processing may be quite different. We have added a short paragraph in the discussion to this effect (pg 19793, ln 24).

4) The authors compare their study site to very different (mainly temperate) systems from other studies that were much bigger. This study was done in a much smaller estuary that seems to show the chemical and morphological characteristics of a river. It would have been interesting to show the relevance of this type of system at the global scale, but the study would need to be replicated. We agree that further similar studies in other systems, as well as targeted studies to specifically test the effect of different environmental variables, are necessary to form overall conclusions regarding MPB carbon fate. The focus of our study, however, was to develop the first budget for MPB carbon processing in subtropical sediments over the longer-term. Given the sampling and analytical effort required to consider as many possible pathways for carbon processing as possible over an extended time period replication in other systems was not feasible. The study is valuable as a stand-alone dataset demonstrating the potential for long-term carbon storage and as the budget developed to describe the processing and fate of MPB carbon within subtropical sediments, which are not well studied. In addition the study incorporates previously unquantified pathways and was done over a longer period than previous similar studies.

5) Also, considering the higher stable isotope values found deeper in the sediment, I wouldn't be surprised if seagrass burial was more relevant to C burial than MPB.

We agree that this is possible, but MPB may still contribute significantly to carbon storage, particularly in systems where seagrass is absent. Our study highlights this as an area worthy of further investigation, as now specified on page 19798, ln 10-11.

Because the data and discussion don't support the hypothesis tested, I will reject this manuscript. The aim of our study was to determine the fate of carbon fixed by MPB in subtropical intertidal sediments. We achieved this aim and believe our manuscript is worthy of publication as it provides evidence to support the novel concept that MPB production may contribute to longerterm carbon storage within coastal sediments, as well as providing the first data of its kind for subtropical sediments, and for some processing pathways for carbon fixed by intertidal MPB. We have altered the last paragraph of the introduction to make the aim of our study more clear. We have specifically stated that quantifying burial was an aim of the study (pg 19776 ln 27), we have removed the sentence from pg 19776 ln 28 – pg 19777 ln 1 "This study provides an opportunity...", and we replaced "hypothesized" with "expected" on pg 19777 ln 4 & 6.

However, the data could be published elsewhere after major revisions. The following points (and other comments in the attached document) will need to be considered and carefully addressed: 1) The title is misleading and needs to be more specific. This is one study in one system, therefore it should not be generalized: "Transformation in a subtropical intertidal sediment" (no plural) We had two sites within the one system, and therefore believe the use of "sediments" (plural) is appropriate. However, we have changed the title to read "…*Potential for* long-term carbon retention…" to make it clear that we are not claiming to have evidence of longer-term "blue carbon" storage.

2) Despite the paper published earlier (Oakes et al. 2010b), the use of the reference to Bellinger et al. (2009) paper was erroneous and PLFA 16:1(n-7) is not MPB-specific. The biomass and uptake calculations need to be redone. Please see the attached document for more details and supportive references.

We thank the reviewer for this comment and attached references. At our study site, based on the average ratio of i+a15:0 to 16:1(n-7) in bacteria-dominated sediments (Rajendran et al. 1993, 1994), we estimate that <2% of the 16:1(n-7) in sediments was derived from bacteria. Cyanobacteria were not present (or at least very scarce) at the study site, but would be part of the microphytobenthos, regardless. We therefore consider 16:1(n-7) to be a suitable MPB biomarker in this case. This has now been explained in section 2.5 of the methods (third paragraph).

3) It will be very important to present the PLFA data and the methods you have used to calculate the different compartment biomasses and uptakes. It is also necessary to present the approach used to calculate the stable isotope signatures (natural abundance) of the different compartments, as well as the conversion of 13C-PLFA to 13C-microorganism. Despite your referring to your previous papers, a lot of the information is in the background and the reader is left to believe and trust. However, my previous point shows that mistakes can be carried over.

The methods used are described fully in our previous papers (cited within the manuscript) which are freely available (open access) but were described only briefly here to reduce overall manuscript length. We have, however, added details regarding the calculation of δ^{13} C values for whole organisms from PLFA δ^{13} C values (section 2.5, first paragraph). PLFAs were not a focus of our paper, and were only used as biomarkers to allow us to determine ¹³C uptake into different compartments. We therefore do not believe that the raw data warrants inclusion in the manuscript as it does not add to the conclusions of our study.

4) You need to take into account the dilution of label for bacteria (and any other heterotroph): while for MPB the DIC pool was 100% labelled, bacteria rely on fresh 13C labelled MPB and MPB-byproducts within a pool of unlabelled labile organic material. You have assessed the standing stock of the different compartments at the start of your experiment. You should use these to estimate the relative contributions based on these dilutions. This also applies to respiration.

We traced a pool of carbon fixed by MPB (labelled with ¹³C) through sediment compartments and transformation processes and created a budget for the transformation and fate of carbon fixed by MPB based on the distribution of this ¹³C at given points in time. E.g. If 10% of the fixed ¹³C was transferred to bacteria, then 10% of the total C fixed by MPB was transferred to bacteria. There is no need to account for label dilution.

Specific comments (page and line number of original manuscript)

(pg 19775, ln 23) The correct reference is Evrard et al. 2010. The paper by Evrard et al. 2012 also looks at ¹³C uptake by MPB into bacteria and fauna and is appropriately cited here. No change has been made.

(19775, 25) The correct reference is Evrard et al. 2008 We have used Evrard et al. 2010 as the citation for ¹³C measurements of DIC, and have removed the Evrard citation for DOC.

(19775, 2) This figure is a conclusive conceptual diagram from the study and therefore does not support this statement. This figure should be the last one but ideally, it could be removed as it doesn't add much to the manuscript, while other data really deserve to be presented.

We have removed the reference to the conceptual diagram from the introduction, so that this figure is now the last one in the manuscript. However, we have left the figure in the manuscript because we believe it helps to clarify and clearly summarise our thoughts about how different processing pathways affect the fate of MPB carbon at our study site.

(19776, 17) The correct reference is Evrard et al. 2008

Both citations are appropriate here, with carbon transfer from MPB studied over 4 days in both cases. We have added the reference to Evrard et al. 2008 and retained the reference to Evrard et al. 2010.

(19776, 9-12) This is true but slightly misleading as the reference suggested here (by the way, they should be cited in this sentence as well) are for very different systems. The temperate areas studied are large tidal estuaries that are orders of magnitude larger and opened to the ocean.

We have added references, as suggested, and have noted that the size of the estuaries and their connection to the ocean can also influence differences in carbon cycling among the systems studied.

(19779, 21 & 25) Prefer: " water sample" or "Sampled water" We have changed to "water sample" to "sampled water", as suggested.

(19780, 15-16) Prefer: "Natural abundance and enriched stable isotope samples were analysed separately to prevent contamination"...

We have changed this sentence as suggested.

(19781, 12) Prefer: "Sediment and fauna samples..." **This has been changed as suggested.**

(19781, 21) "the" is missing in front of "addition" **This has been corrected.**

(19781, 3-5) What do you mean by "highly mobile": they couldn't be caught or they were washed away? This is rather unfortunate as I would expect that herbivory and secondary consumption would be significant to MPB C processing. The "focus" on smaller fauna is biased toward fauna which diet is likely relying on smaller particles and/or dissolved organic carbon). However, during flood events, the reworking of the sediment will remove a large fraction of the large fauna and the meiofauna if any, will be the most resilient or the first one to recolonize the habitat.

Species that were highly mobile would not have remained within the labelled area for any length of time, and containment would have drastically altered their behaviour and would not have given a reliable indication of their contribution to carbon processing. The paragraph on pg 19781 ln 3-5 has been re-worded to clarify this.

The low abundance of benthic macrofauna at the site makes it is unlikely that herbivory by these taxa contributed much to MPB processing. The lack of macrofauna and relative

abundance of meiofauna is typical of this site and other tropical systems (Purwoko and Wolff 2008), probably reflecting the highly variable environmental conditions, as hypothesised by this reviewer, and this is now explained in the manuscript (pg 19793 ln24).

(19781, 6) One replicate means duplicate samples (n=2). n=1 means no replicate. Which one is it? **This has now been clarified (n=1) on pg 19781 ln 5-6.**

(19781, 16-17) only sediment got the unacidified treatment?

No change has been made in response to this comment. We calculated the C:N ratio of sediment organic matter as part of the site characterisation and needed to analyse an unacidified sample of sediment to determine %N for this calculation. Both foraminifera species had calcareous tests and were therefore acidified to remove this inorganic carbon.

(19782, 26) .../(SA x t) reads better **This has been changed, as suggested.**

(19782, 14-15)16:1(n-7) PLFA likely had the biggest peak but unfortunately, this PLFA is not specific of MPB and the reference found in Oakes 2010b (Bellinger et al. 2009) is not appropriately used. This reference clearly states that this PLFA is found cyano and gram bacteria. Here are other references: Boschker et al. 2005 and Boschker et al. 1998 (*abbreviated citations provided here*). We thank the reviewer for this comment and associated references. As described above, we estimate that <2% of the 16:1(n-7) in sediments was derived from bacteria. Cyanobacteria would be part of the microphytobenthos, regardless, but were not present. We therefore consider 16:1(n-7) to be a suitable MPB biomarker in this case. This has now been explained in section 2.5 of the methods (third paragraph).

(19782, 24) "Total flux of excess": This terminology is confusing and it's inconsistent with the terminology used on 1.6 (you should then call it flux of uptake). This new terminology basically means flux of increase and doesn't mean much. It would be more precise to just call it 13C or uptake rate, which is exactly what is being measured.

The terminology used is correct and is consistent with that used elsewhere in the paper. Throughout the paper we describe the flux of ¹³C that is in excess of that which would occur naturally (i.e. without addition of label). Therefore, it is not simply the flux of ¹³C that was measured, but the flux of *excess* ¹³C. No change has been made.

(19783, 24-25) Prefer: "..., another rain event increased the flow..." **This has been changed, as suggested.**

(19783, 7-9) The area underneath a function's curve is the integral of that function. It would probably be more precise to just say: "total amounts were calculated from discrete flux values integrated over the interpolated time periods"

The description is not incorrect and is simpler, so this has not been changed.

(19784, 4-7) This comment and reference belong to the discussion and are repeated there anyway. However, this clearly shows the discrepancy between this type of system and the ones studied in temperate areas. Considering the scales, I would be cautious when generalizing to "intertidal subtropical sediments"

We believe that it is important to be clear that the conditions we describe are not atypical, and this information (which alludes specifically to the system studied) therefore belongs in the methods section. We have removed some of the similar content from the discussion (pg 19793, ln 27-29).

Our system is typical of subtropical systems, and similar processes may well occur in these other episodically flushed systems as well. However, we have been careful not to 'overstate our case', and throughout the discussion we clearly allude only to the 'potential' of subtropical sediments, and what 'may' occur. We agree that further studies are warranted, and we note this in the concluding paragraph of the manuscript.

(19784, 23-27) The sampling occurs after a rain/flood event and is biased towards the meiobenthos. It's not a problem but what are remaining 38%? I think this would help indicate what species get washed away or buried after the rain event.

As described on pg 19784 ln 23, fauna in the sediment consisted of 51 species, which were primarily foraminiferans.

(19785, 1) The 1% contribution basically says that they are negligible. They could very well be pooled with the uncharacterized fraction.

We can see the referee's point. However, in a previous study (Oakes et al. 2012) one species of foraminifera accounted for ~10-30% of the ¹³C within sediment organic carbon, despite representing only 0.8 -3.8% of the sediment organic carbon biomass. This demonstrates that although the species we analysed had low biomass, if they had acquired substantial label they may still have played an important role in processing of carbon fixed by MPB. For this reason, we believe it is better to show this data than to simply combine the fauna with the truly uncharacterized fraction.

(19785, 5-8) This uncharacterised fraction has the typical stable isotope signature of terrigenous material which has been deposited in huge amounts on the flat and it is consistent with the fraction of fine sediment found closer to the sediment surface.

This likely contribution of terrigenous material, as suggested by the fine surface sediments, is now noted on page 19789, ln 28.

(19785, 11-13) How did you measure microphytobenthos and bacteria d13C? Did you account for fractionation between biomass and PLFA?

We calculated $\delta^{13}C$ values for bacteria and MPB taking into account fractionation between biomass and PLFA. These corrections are described in the papers cited, which are freely available (open access). No change has been made.

(19785, 15-17) This clearly suggest a recent terrigenous deposition event, probably following the previous flood. The higher 13C signature underneath suggest buried seagrass detritus and/or MPB. We have now specifically noted the possible contribution of terrigenous sediment deposition to the uncharacterised OC (pg 19789 ln 28). We had already noted that OC in deeper sediments may be derived from buried MPB or seagrass carbon (pg 19790 ln 6-7), so no change has been made in response to this latter comment.

(19785, 20) This is yet another term to describe 13C uptake No change has been made, as we do not believe the sentence is unclear.

(19785, 24) Prefer: "within 4h after label addition" **This has been changed as suggested.**

(19785, 25-26) Maybe this and the following sentence should be kept to the discussion with reference. It is our opinion that it is important that the uptake rates that are calculated and presented in the results section are viewed in context, i.e. it is important to note that they are underestimated because of likely carbon limitation. For this reason, this sentence has been left in the results section.

(19786, 11) "a maximum" instead of "for a peak" **This has been changed, as suggested.**

(19786, 8-9) These calculations need to be redone without 16:1(n7) as commented in the methods. Despite the absence of cyanobacteria and in the context of an estuarine environment with several

flood events and with a strong fresh water influence, there are quite a few types of gram bacteria that could account for the 16:1(n7).

We have not revised the calculations, as we estimate the contribution of bacteria to the 16:1(n-7) peak to be <2%, as now described in section 2.5, third paragraph. Furthermore, owing to the lower label uptake by bacteria (based on δ^{13} C values of i+a15:0), any 16:1(n-7) contributed would have a lower δ^{13} C value than algal-derived 16:1(n-7). This lower δ^{13} C value would offset any increase in peak area due in inclusion of bacteria-derived 16:1(n-7) in our calculations of the ¹³C content of MPB.

(19787, 1-28) for consistency sake: a negative value for uptake means an export or loss and the other way around... The or + should be used with the term flux to give the direction (import or export in the sediment).

This has been corrected.

(19788, 5-23) The biomass to productivity ratio might be overestimated because of the overestimated biomass from PLFA. I think the PLFA data needs to appear somewhere in a table or figure to get an idea of the composition and stable isotope values. Too much data is in the background. There is definitely a good opportunity to use a Chemtax approach to derive the MPB composition. See the following and please also note that 16:1(n7) is not specific of microalgae. Although the following references are for phytoplankton, the same approach can be used to estimate MPB. See: Dijkman et al. 2009, Dijkman & Kromkamp 2006 (*abbreviated citations provided here*).

As aforementioned, we do not believe that biomass has been estimated due to the use of 16:1(n-7). However, we have now included an estimate of the error in biomass of bacteria and MPB that may have arisen due to variations PLFA composition (section 2.5, paragraph 4). The use of Chemtax would be an interesting use of a similar data set, but we do not believe we can make effective use of Chemtax with the data we have, as some PLFAs had insufficient separation/peak height, and we do not have sufficient data on the specific groups of algae present (beyond knowing that the MPB was mainly comprised of various diatoms) and their likely lipid compositions. Furthermore, our focus was on the processing and fate of the carbon fixed by the MPB community, rather than specific groups of MPB.

(19789, 5-6) Do you account for the production of EPS as well? You have measured 13CDOC, is it included here?

As stated in the introduction (first paragraph), DOC fluxes include EPS. We assume that the referee is querying whether or not we allowed for release of EPS by MPB in our calculations of productivity. The answer to this is that our productivity estimates were based on ¹³C uptake into total sediment organic carbon, which would include any EPS which had been produced, but we would not have been able to account for any loss of EPS from the sediments during the first inundation, as we did not measure DOC fluxes for this time. However, given that we accounted for 100% of the added carbon at the time we first sampled, there must have been negligible loss of ¹³C from sediments as EPS beforehand. This fits with our conclusion that there was a low rate of EPS production at this study site (page 19791, ln 26).

(19789, 19-20) Although I imagine how this was calculated, it is important to give the details of your calculations, especially that: 1) MPB could be overestimated because you used 16:1(n7), As aforementioned, we do not believe that MPB biomass was overestimated, based on an estimated contribution of bacteria to 16:1(n-7) of <2% (now noted in section 2.5, paragraph 3).

(19789, 19-20) 2) There is a discrepancy between PLFA 13C signature and MPB as a whole 13C signature. Did you use a 13C mass balance of 16:1(n7) and 20:5(n3)? We used only 16:1(n-7), as described in the cited paper. We have now included details of this calculation in section 2.5, paragraph 1.

3) Here again, some data is in the background and needs to be taken to the foreground in a table or figure and the calculations need to be described in the Methods.

Isotope mixing calculations used values shown in table 1. This has now been detailed on page 19789, ln 19-20.

(19790, 3-9) This is likely seagrass and shows that seagrass C storage might be important We do not dispute that seagrass burial could be important for carbon storage at the site, but this does not mean that MPB carbon storage is not also important. We noted the possible contribution of seagrass to buried organic carbon in the original manuscript (pg 19790, ln 7).