

*We would like to thank the editor and referees for their valuable comments. Below we address all comments point-by-point.*

### **Referee #1**

Comment from referee: L16 Check repeated text

*Author's response: The abstract has been modified with this line removed.*

Comment from referee: L71-72 This sentence is out of place

*Author's response: This sentence has been moved up. L68*

Comment from referee: Line 78 and various other place throughout manuscript - temperature and tropical mangroves? I am assuming the authors mean temperate (also line 84 but check throughout MS)

*Author's response: Apologies. We meant temperate. The mistake was corrected throughout the manuscript.*

Comment from referee: Line 108 "hydrodynamic exposure" what is meant by this? In my original review I requested information on hydrodynamics such as current velocities, inundation time etc, this seems to be the response of the authors to this request but the term is not defined

*Author's response: This sentence has been modified to include how we characterised sheltered vs exposed. We note that this is a subjective classification as we did not record current velocity or inundation time.*

*"hydrodynamic conditions (sheltered: protected from direct wind and wave action, generally located in the upper reaches of the estuary; exposed: exposed to wind and wave action, generally located in the lower reaches of the estuary)". L114*

Comment from referee: L117 The shading experiment is flawed in 2 ways - 1) The measurements were not undertaken over the same sediment (they were adjacent), and considering the large within site variability, it is no wonder there was no significant difference! 2) The experiments were undertaken in the "intact" mangroves. This is the location where the effect of MPB on measured fluxes is likely the lowest due to the lower light penetration under the mangrove canopy. This experiment should have been undertaken in the cleared environments where presumably benthic photosynthetic rates would have been highest, therefore the artefact associated with continuing CO<sub>2</sub> uptake in the chambers would have been highest.

*Author's response:*

- 1. We acknowledge that the shading experiment has its limitations and we have expanded on this in the discussion. We also note that repeating the experiment on the same sediment at a different time point is potentially confounded by temporal variation. We note that the shading structure itself may also impact flux through factors other than shade (i.e. changing atmospheric pressure or subtle effects on temperature, changing the behaviour of shaded macrofauna etc).*

*“We note that spatial variation in sediment CO<sub>2</sub> efflux may partly explain the lack of a pre-shading effect. Further, our shading experiment was restricted to an intact mangrove forest site. A study by Granek and Ruttenberg (2008) investigating the effect of mangrove clearing on abiotic and biotic factors in Panama showed that cleared mangrove sediments are exposed to higher light levels. Thus the activity and the response of photosynthesising biofilm communities to pre-shading may differ in cleared mangrove forests.” L430*

- 2. The experiment was undertaken in intact mangroves because chlorophyll  $\alpha$  values were generally higher. Higher sediment chlorophyll  $\alpha$  contents in mangrove forest sediments suggest increased photosynthetic activity (Leopold et al., 2013; Bishop et al., 2007). Uptake was also observed within intact mangrove at Hatea showing this was not limited to clearance sites.*

Comment from referee: L150 30 seconds between biofilm removal and flux measurements - no wonder fluxes were significantly higher, need time to re-establish diffusion gradients.

*Author’s response: Given the changes in abiotic conditions due to tidal fluctuations we did not run a series of measurements following biofilm removal. However, we agree that this would have been required to determine the magnitude and length of time any potential effect occurs. We have acknowledged the effect of surface sediment removal on diffusion gradients in the discussion.*

*“It is also possible that the increase in CO<sub>2</sub> efflux following biofilm removal is related to the modification of sediment profiles, changing the oxygen distribution and anoxic/oxic interface, and resulting in increasing diffusion gradients (Kristensen, 2000).” L454*

Comment from referee: L152 As written the second term of this equation is not correct, should be (P.V)/(R.T.A)

*Author’s response: We corrected the equation. L164*

Comment from referee: L154 Regression of concentration vs time should give units of ppm/second

*Author’s response: This section has been modified. L166*

Comment from referee: L168 "dionized"?

*Author’s response: Changed to “distilled”. L181*

Comment from referee: L168 - What temperature was the hotplate? Not too hot I hope as OM degradation will occur if temps are raised too high!

*Author’s response: This section has been modified as follows:*

*“Briefly, 300 mg sediment was mixed with 0.5 ml deionized water and 1.5 ml of 20% HCl and then dried on a hot plate at 60°C.” L181*

Comment from referee: L178 How was sample stored for the 1 month prior to analysis? Chl  $\alpha$  requires very specific storage to prevent degradation

*Author's response: This section has been modified as follows:*

*"After collection the samples were frozen and stored in the dark before analysis" L175*

Comment from referee: L216 See comments above - you cannot do a paired t-test on samples that are technically not the same site

*Author's response: We re-analysed the data using Mann-Whitney Rank Sum Test. We note that the significance level did not change.*

Comment from referee: L251 - Visual analysis? Any method to determine these cut-offs (eg. cluster analysis). Otherwise this kind of separation by non-statistical methods opens up the possibility of "p-hacking" whereby the group classification is determined based on producing significant differences between the groups. I would like to see a more quantitative separation of these groups.

*Author's response: We have updated the paragraph to better explain the process of grouping sites and removed references to visual analysis.*

*"Individual sites were grouped based on whether CO<sub>2</sub> efflux exceeded ('high efflux group') or was below ('low efflux group') the mean CO<sub>2</sub> efflux rate for intact mangrove forests (168.5  $\pm$  45.8 mmol m<sup>-2</sup> d<sup>-1</sup>), to determine whether site characteristics were significantly different between high and low efflux groups." L268*

Comment from referee: L260 - Again some method other than "visual analysis" to separate groups is required.

*Author's response: We modified this paragraph. As above. L 268*

Comment from referee: L305-308 If microbial communities drive the higher fluxes at the high efflux sites, why were the fluxes higher when the biofilm was removed? This argument does not fit the biofilm removal story

*Author's response: We assume that the efflux is driven by different microbial communities. CO<sub>2</sub> uptake due to photosynthesising biofilm communities (which might offset the total efflux) vs CO<sub>2</sub> efflux due to microbial respiration of sediment microbial communities and heterotrophic biofilm communities following biofilm removal. The heterotrophic respiration of biofilm communities may differ significantly due to the density or activity of the community. However, once this biofilm/surface sediment is removed, the flux significantly increases at all sites.*

Comment from referee: L316-317 True - but you do not have any light data to discuss this

*Author's response: We agree that without light data we are limited in our interpretation of the role of photosynthesising communities. We have modified the statement.*

*"Respiration from heterotrophic biofilm communities also contribute a considerable proportion to total CO<sub>2</sub> efflux from mangrove sediments, as shown in a New Caledonian*

*Avicennia marina* forest (Leopold et al., 2013). High sediment chlorophyll  $\alpha$  concentrations and the presence of algal mats characterising the intact ‘high efflux sites’ suggests that respiration by heterotrophic biofilm communities may be a significant contributor to  $\text{CO}_2$  efflux (Decho, 2000).” L341

Comment from referee: L332 - What is meant by exposure?

*Author’s response: We have modified this sentence.*

*“We note that all sediment  $\text{CO}_2$  efflux measurements in this study were made at low to mid-tide while surface sediments were exposed to air, and likely over-estimate maximum efflux rates across a tidal cycle. Mangrove sediment  $\text{CO}_2$  efflux during low tide can be up to 40% greater than during tidal immersion as molecular diffusion of  $\text{CO}_2$  is faster when sediments are aerated and the surface area for aerobic respiration and chemical oxidation increases (Alongi, 2009).” L351*

Comment from referee: L344-348 - Any data on nutrients? If not this is a bit speculative. Also high clay content is usually a covariate with SOC because of hydrodynamics - i.e. where there is depositional conditions (i.e. low current velocity), both clays and OM settle out. While there is some effect of surface charge on OM adsorption - generally clay content and SOC are just covariates

*Author’s response: We have modified the paragraph as follows.*

*“High clay content and sediment organic C concentration characterised the ‘high efflux sites’. Spatial covariation of clay and organic C has been found in terrestrial soils (Davidson, 1995) but also applies to coastal sediments (Hu et al., 2006). For example, both clay and organic C settle out on the sediment surface in areas where there is low current velocity. Clay content has been shown to be associated with higher  $\text{CO}_2$  efflux in tropical mangrove forests (Leopold et al., 2013; Chen et al., 2010; Chen et al., 2012; Chen et al., 2014).” L394*

*We also note that sediment N concentration was higher in the higher efflux group, as mentioned in the results.*

Comment from referee: L386-388 See comment above regarding the problems with the shading experiment

*Author’s response: See response above.*

Comment from referee: L389-391 This is not really plausible unless the  $\text{CO}_2$  concentration in the sediments is lower than ambient air - which is not likely

*Author’s response: We have removed this statement.*

Comment from referee: L403-404 I do not believe that chemosynthetic  $\text{CO}_2$  uptake can exceed respiration in these organic rich sediments

*Author's response: We have rephrased this sentence to suggest that chemosynthetic CO<sub>2</sub> uptake "may" contribute to the CO<sub>2</sub> uptake observed, to avoid inferences that they are solely capable of the uptake rates observed.*

*"Chemoautotrophs have also been shown to fix C in intertidal sediment under dark conditions (Boschker et al., 2014; Lenk et al., 2011) and may contribute to the decrease in CO<sub>2</sub> concentration measured in the dark chamber. In particular at the interface of aerobic and anaerobic zones where large amounts of reduced compounds, such as sulphur, accumulate (Santoro et al., 2013; Boschker et al., 2014; Thomsen and Kristensen, 1997; Lenk et al., 2011)." L440*

## Referee #2

Comment from referee: The manuscript has improved very much by the revision. I like that the authors took the time to examine the effect of pre-shading. To me it looks like there is an effect, although the statistics could not prove it – the CO<sub>2</sub> flux for intact sediment almost doubled. In any case, the pre-darkening could not explain the reason for the large difference when the surface 2 mm sediment is removed. It might be due to the exposure of a steeper gradient driving the higher flux. Anyway, I am still not sure I understand why this artificial manipulation was done. What does it tell us? The authors argue in the discussion (p. 15) that one reason for the observed difference is the homogenization of surface sediment by the removal procedure. This is quite likely and then the results depend on the type of handling – and as such do not tell us anything about the system. They also argue that chemoautotrophs in the biofilms fix carbon and are responsible for the lower flux in intact sediment. I agree that this to some extent is true, but do not explain the results measured here – which in some cases even provided CO<sub>2</sub> uptake without removal of the biofilm. This is impossible under steady state conditions. If the chemoautotrophs are driven by sulfide, then it will require oxidation of about 5 sulfides to fix one CO<sub>2</sub>. The reduction of sulfate to sulfide by sulfate reduction on the other hand will gain two CO<sub>2</sub>. From this calculation it is obvious that the role of chemoautotrophs for the CO<sub>2</sub> flux is not as important as stated.

### *Author's response:*

*Shading experiment. We conducted the shading experiment to test if CO<sub>2</sub> uptake can be explained by the photosynthetic activity of autotroph biofilm communities as suggested by previous studies (Leopold et al., 2015). Although the shading experiment did not support this hypothesis we can't exclude this being the case as our experiment was limited to one site and spatial variability was high. We modified the discussion accordingly. We acknowledge the limitations of the pre-shading experiment. We did not control for potential impacts of shading on sediment and air temperature, atmospheric pressure, changes in CO<sub>2</sub> concentrations or the behaviour of fauna. All of these factors are likely to influence efflux rates and confound comparisons, unrelated to the influence of lagged photosynthesis. While also not significant, the difference between the two biofilm removed treatments looks similar to the difference between the two biofilm intact treatments, which is unrelated to lagged photosynthetic processes as the biofilm is removed.*

*Biofilm removal. We wanted to investigate the effect of biofilm on sediment CO<sub>2</sub> efflux during dark chamber measurements and to explore whether the intact biofilm was reducing or increasing CO<sub>2</sub> efflux, and whether this varied among sites. We were also interested in whether the clearance of mangrove had an impact on the function of the biofilm and what happens following sediment disturbance (e.g. during clearances). We agree that there are limitations associated with scraping off the top sediment, including the potential modification of sediment profiles, and have mentioned this in the manuscript.*

*“Sediment CO<sub>2</sub> efflux was consistently higher across both intact and cleared mangrove sites following the removal of the top 2 mm of sediment. Other studies have suggested that the surface biofilm may act as a barrier to the flow of CO<sub>2</sub> from deeper sediment, which when removed results in a rapid increase in CO<sub>2</sub> efflux (Leopold et al., 2015; Leopold et al., 2013). It is also possible that the increase in CO<sub>2</sub> efflux following biofilm removal is related to the modification of sediment profiles, changing the oxygen distribution and anoxic/oxic interface, and resulting in increasing diffusion gradients (Kristensen, 2000). Our findings demonstrate that relatively small disturbances to the sediment column such as biofilm removal have significant impacts on sediment CO<sub>2</sub> efflux. This illustrates the complexity of processes*

*influencing sediment CO<sub>2</sub> efflux in coastal wetlands and generates further questions (for example, what is the duration of this effect? Does the magnitude of the effect change depending on the clearance method? What effect does wind or wave disturbance have on efflux rates?).” L450*

*We agree that the activity of chemoautotrophs in the surface biofilm do not explain the uptake of CO<sub>2</sub> observed at some sites. We have modified this section of the manuscript to make it clear that they may contribute to the uptake rates observed but are not solely responsible.*

*“Chemoautotrophs have also been shown to fix C in intertidal sediment under dark conditions (Boschker et al., 2014; Lenk et al., 2011) and may contribute to the decrease in CO<sub>2</sub> concentration measured in the dark chamber. In particular at the interface of aerobic and anaerobic zones where large amounts of reduced compounds, such as sulphur, accumulate (Santoro et al., 2013; Boschker et al., 2014; Thomsen and Kristensen, 1997; Lenk et al., 2011).” L440*

Comment from referee: I am also somewhat concerned about the lack of information about number of pneumatophores and burrows inside the measuring chamber. The authors are aware of the problem, but it would be nice to see if there are any correlations of flux results with the presence of these biogenic structures.

*Author’s response: Pneumatophore abundance and crab hole density were recorded at the cleared sites. However, we did not find significant correlations between sediment CO<sub>2</sub> efflux and crab hole and pneumatophore abundance.*

Comment from referee: I now find the manuscript acceptable for publication when revised according to my concerns above and the corrections listed below.

Specific points:

Abstract:

Comment from referee: Line 11: Delete “temperate”

*Author’s response: Modified as suggested.*

Comment from referee: Line 16-17: There is a copy-paste error here as the same text is shown twice.

*Author’s response: Modified as suggested.*

Introduction:

Comment from referee: Line 33: Change to “These forests are subject to....”

*Author’s response: Modified as suggested.*

Comment from referee: Line 36: Change to “....temperate mangrove sediments....”

*Author’s response: Modified as suggested.*

Comment from referee: Line 47: Change to "...expansion of mangrove forests..."

*Author's response: Modified as suggested.*

Comment from referee: Line 77-78: Change to "Lovelock (2008) found a positive correlation between leaf area index and sediment CO<sub>2</sub> flux in temperate and tropical mangrove forests."

*Author's response: Modified as suggested.*

Materials and methods:

Comment from referee: Line 124 & 127: It reads better writing "removal"

*Author's response: Modified as suggested.*

Comment from referee: Line 153-154: How can the units of the regression slope be  $\mu\text{mol mol}^{-1}$  when the regression describes change in CO<sub>2</sub> concentration over time? It must be  $\mu\text{mol s}^{-1}$

*Author's response: Changed accordingly.*

Comment from referee: Line 166: Why was it only a subset of samples?

*Author's response: Due to the number of samples which needed to be processed we acidified a subset of the total samples. We note that we found a significant relationship between acidified and non-acidified samples (mentioned below):*

*"A linear regression function between total C and organic C ( $r^2 = 0.98$ ,  $p < 0.001$ ) was used to calculate organic C concentrations of non-treated samples. L183*

Comment from referee: Line 202: It is not clear if this equation was used for all trees in Mangere 1 and Hatea 1, or if it was only trees exceeding the maximum height (248 cm) of equation 3.

*Author's response: This equation was used for all trees at Mangere 1 and Hatea 1. The sentence has been modified to make this clear. L217*

Comment from referee: Line 210: It seems not logic than root biomass was not measured in mangrove sites. Here these structures must be expected to be most important!

*Author's response: We acknowledge that cores and quadrat measurements would have been useful for the interpretation of our findings in the intact mangroves. The measurements were restricted to cleared mangrove sites as greater variation in these measures was expected within cleared sites.*

Comment from referee: Line 243 and elsewhere: The unit for fluxes given per second is not standard in these types of studies. In fact at line 281, the authors make the conversion to per day for comparative purpose. This also makes Table 2 somewhat strange because the same data are given in two columns, one per day and one per second. Please convert the results to per day throughout. Then Table 2 will only consist of one data column – and readers familiar



with the per day notation need not do the calculation to get rates they can compare with others.

*Author's response: We converted the results to  $\text{mmol m}^{-2} \text{d}^{-1}$  as suggested.*

Comment from referee: Line 251-252: Are these clusters shown anywhere?

*Author's response: As explained in more detail in our response to Reviewer #1, we have updated the paragraph to better explain the process of grouping sites and removed references to visual analysis. This grouping was based on whether individual site means were higher or lower than the overall mean for intact mangrove forest (or cleared mangrove forest). L268*

## Discussion

Comment from referee: Line 288-289: Of this reason it would be nice with some data on numbers of burrows and pneumatophores.

*Author's response: Pneumatophore abundance and crab hole density were recorded at the cleared sites. However, we did not find significant correlations between sediment  $\text{CO}_2$  efflux and crab hole and pneumatophore abundance*

Comment from referee: Line 313: Measurements were made at low to mid-tide. I suppose that the sediment was still air exposed?

*Author's response: Yes. We have modified the sentence as follows:*

*“We note that all sediment  $\text{CO}_2$  efflux measurements in this study were made at low to mid-tide while surface sediments were exposed to air, and likely over-estimate maximum efflux rates across a tidal cycle. Mangrove sediment  $\text{CO}_2$  efflux during low tide can be up to 40% greater than during tidal immersion as molecular diffusion of  $\text{CO}_2$  is faster when sediments are aerated and the surface area for aerobic respiration and chemical oxidation increases (Alongi, 2009).” L351*

Comment from referee: Line 334-340: Do you find any indication of lower organic quality in older cleared sites?

*Author's response: We did not look at changes in carbon quality over time, although this is an important consideration and will be taken on board for future work. We expect that the quality of sediment carbon is likely to change variably following mangrove clearance, depending on factors such as faunal activity or the relative contribution of carbon from external sources such as mulched material.*

*“Decomposition and thus sediment  $\text{CO}_2$  efflux rates are not only controlled by the amount of C and N but also by the quality of the substrate and activity of the decomposer community (Kristensen, 2000). As C quality was not measured in this study it remains unknown whether the observed positive correlation between sediment organic C concentration and sediment  $\text{CO}_2$  efflux is driven by C quality or quantity.” L388*

Comment from referee: Line 346: Was this accumulation of carbon due to sedimentation or what?

*Author's response: We have included the following sentences in the discussion.*

*“The main sources of organic C in intact mangrove sediments are litter and root material and suspended matter from other terrestrial and estuarine sources (Bouillon et al., 2003). The relative contribution of each source has been shown to vary considerably depending on site characteristics and histories (Bouillon et al., 2003).” L327*

Comment from referee: Line 349: Change to “...particularly in dwarf/stunted mangrove forests...”

*Author’s response: This section has been revised*

Comment from referee: Line 350-351: Is this correlation shown anywhere?

*Author’s response: Correlations were tested but not included in the manuscript. We have revised this sentence and referred to regression coefficients rather than correlations. L381*

Comment from referee: Line 361-370: Do these differences in organic carbon be due to sedimentation of particles from the tidal water or what? It is not clear from where the enrichment originates.

*Author’s response: As mentioned above regarding the origin of carbon in mangrove systems. L327*

Comment from referee: Line 386-388: True, but there seems to be a marginally significant effect.

*Author’s response: As mentioned above.*

Comment from referee: Line 389-397: I am not fully satisfied by the explanations for the strongly increased flux after removing the upper 2 mm of the sediment. The authors could mention displaced profiles and thus strongly increased gradients.

*Author’s response: We have included the following in the discussion:*

*“It is also possible that the increase in CO<sub>2</sub> efflux following biofilm removal is related to the modification of sediment profiles, changing the oxygen distribution and anoxic/oxic interface, and resulting in increasing diffusion gradients (Kristensen, 2000).” L454*

Comment from referee: Line 398: It is true that chemoautotrophs fix carbon, but they can never fix more than generated by heterotrophic processes in the entire sediment column.

*Author’s response: We have rephrased this sentence to suggest that they may contribute to the CO<sub>2</sub> uptake observed, to avoid inferences that they are solely capable of the uptake rates observed.*

*“Chemoautotrophs have also been shown to fix C in intertidal sediment under dark conditions (Boschker et al., 2014; Lenk et al., 2011) and may contribute to the decrease in CO<sub>2</sub> concentration measured in the dark chamber. In particular at the interface of aerobic and anaerobic zones where large amounts of reduced compounds, such as sulphur, accumulate (Santoro et al., 2013; Boschker et al., 2014; Thomsen and Kristensen, 1997; Lenk et al., 2011).” L440*

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