

Overview comment (to all referees)

We would like to acknowledge the helpful comments received by the referees. Here we address two of the main concerns expressed by the referees. We note that the referees expressed a recommendation that while the manuscript contained a large amount of valuable information, it should focus on the main factors influencing CO₂ efflux. In addition the referees asked for a more detailed description of the methods. We have addressed these concerns and suggestions by:

- Omitted the tidal flat data to concentrate on CO₂ efflux from intact and cleared mangrove forest sites and the main factors influencing the sediment CO₂ efflux.
- Removed the macrofaunal data
- We have reassessed the criteria for including flux data. In the revised version only fluxes where the r^2 of the linear regression (increase of CO₂ concentration vs time) exceeds 0.8. In general, r^2 values of less than 0.8 occurred at sites where there was minimal change in CO₂ efflux, typically less than $\pm 0.4 \mu\text{mol m}^{-2} \text{s}^{-1}$. While it is possible that the flux at these sites exhibits a non-linear trend, we have removed them to in order to strengthen the interpretation of the remaining dataset.
- This resulted in a decline in the number of clearance sites from 40 to 23, and intact mangrove forest sites from 18 to 13.
- While working on the calculations we identified an error in the CO₂ efflux calculation script (the chamber volume was overestimated by about 40 %) and we re-calculated all sediment CO₂ efflux values, re-did all related statistical tests, corrected the tables and figures.

The second point raised by referee#3 was in regards to the procedure of the CO₂ flux measurements, i.e. the possible continuation of photosynthesis if measurements were made immediately after the chamber deployment. Based on this we undertook additional measurements to test the impact of pre shading the sediment for > 30 minutes prior to dark CO₂ efflux measurements. We selected an existing location (Hatea 1) where CO₂ uptake had previously been measured. The manuscript has been modified to include the results of this experiment.

We compared control and biofilm removed measurements using identical methodology to that described in the manuscript. Relevant sections are included below:

2.3.1 Pre-shading the sediment

Frames (0.5 m²) were located approximately 20 cm above the sediment surface. The frame was completely covered by layered cloth to exclude light penetration. At site Hatea 1, three frames were deployed throughout the mangrove forest, at least 10 m from each other and the mangrove edge. After 30 minutes of shading, two CO₂ efflux measurements using a dark

respiration chamber were conducted at different locations within the 0.5 m² area, before and after the removal of the surface biofilm. The biofilm (top ~2 mm of surface sediment) was scraped off using a spatula. Biofilm removed measurements were collected immediately following biofilm intact measurements in the identical location. Corresponding dark CO₂ efflux measurements were also conducted at locations that had not been pre-shaded (control) adjacent to each shaded measurement, as well as corresponding biofilm removed measurements to account for heterogeneity in sediment conditions.

2.3.2 Sediment CO₂ efflux from intact and cleared temperate mangrove

Sediment CO₂ efflux was measured in the centre of the cleared sites at three randomly selected locations. Locations in the intact mangrove forest were > 10 m from the cleared areas. No pre-shading of the sediment was undertaken prior to measurements.

The sediment CO₂ efflux was measured at low tide, between 8 am and 6 pm local time, using an infrared CO₂ analyser (Environmental Gas Monitor (EGM-4) with a dark sediment respiration chamber (SRC-1, PP Systems Ltd., Amesbury, MA, USA). Using a dark chamber prevents the photosynthetic activity of benthic microbial communities which results in the uptake of CO₂. A PVC collar (10 cm height) was attached to the base of the respiration chamber to protect the chamber from potential flooding. The collar was inserted approximately 5 mm into the sediment, avoiding damage to surface roots. Sediment within the chamber included crab burrows and pneumatophores < 7 cm which fit within the respiration chamber. The sediment area covered by each chamber was 0.00785 m². Chamber height was measured during each measurement as collar insertion varied based on sediment characteristics. Total chamber volume varied between 1.72 and 1.98 l depending on the depth of collar insertion. The CO₂ concentration in the chamber was measured at 5 second intervals over a 90 second period. Air and sediment temperature (Novel Ways temperature probe) and moisture (CS620, Campbell Scientific, Logan, UT, USA) to a depth of 12 cm was measured with each CO₂ efflux measurement.

In addition to measuring CO₂ efflux in intact (undisturbed) sediment, sediment CO₂ efflux was re-measured at the same location after the removal of the surface biofilm. Measurements were made within 30 seconds following the removal of the surface biofilm.

Sediment CO₂ efflux was calculated from linear regression of the CO₂ concentration within the chamber over time. Only regressions with r^2 values ≥ 0.8 were used for flux calculations.

The sediment CO₂ efflux rate was calculated as follows.

$$\text{CO}_2 \text{ flux } (\mu\text{mol m}^{-2} \text{ s}^{-1}) = (\Delta\text{CO}_2/\Delta t) \times (P \times V/R \times T \times A) \quad (1)$$

Where $\Delta\text{CO}_2/\Delta t$ is the change in CO_2 concentration over time, based on the slope of the linear regression ($\mu\text{mol mol}^{-1}$), t is time (s), P is the atmospheric pressure (Pa), V is the volume of the chamber including collar (m^3), A is the surface area covered by each chamber (0.007854 m^2), T is the temperature (K), R is the ideal gas constant, $8.20528 \text{ m}^3 \text{ PaK}^{-1} \text{ mol}^{-1}$.

We note that as part of a separate study we also undertook similar testing within intact mangrove at a new location (Whangateau 2), with similar results which we include in the response to referees but not the manuscript. A total of 18 measurements were collected for each treatment at Whangateau 2 (control biofilm intact, and control biofilm removed, shaded biofilm intact, shaded biofilm removed).

Statistical analysis used:

A Shapiro-Wilk test was used to test normality. As data conformed to normality, paired t-tests were used to determine significant differences ($p < 0.05$) in shaded and control measurements of sediment CO_2 efflux within intact mangrove at Hatea 1.

Results of the additional testing at Hatea 1:

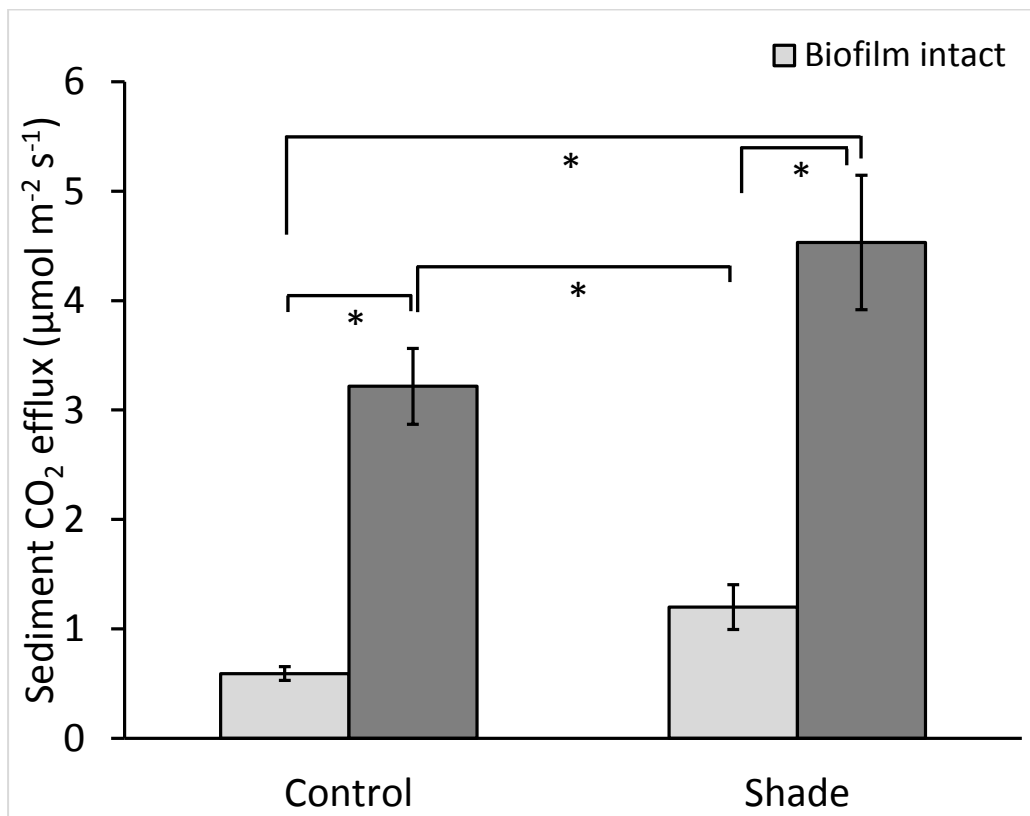


Figure 1. Mean sediment (\pm SE) CO_2 efflux ($\mu\text{mol m}^{-2} \text{ s}^{-1}$) before and after surface biofilm was removed, from control ($n = 6$), and pre-shaded sediment ($n = 6$) at intact mangrove site Hatea 1. *significant difference ($p < 0.05$)

No significant difference ($p > 0.05$) was detected in mean CO₂ efflux between shaded and control treatments (Figure 2). Removing the surface biofilm resulted in significantly higher CO₂ efflux ($p < 0.05$) for both shaded and control treatments (Figure 2).

Results of the additional testing at Whangateau 2:

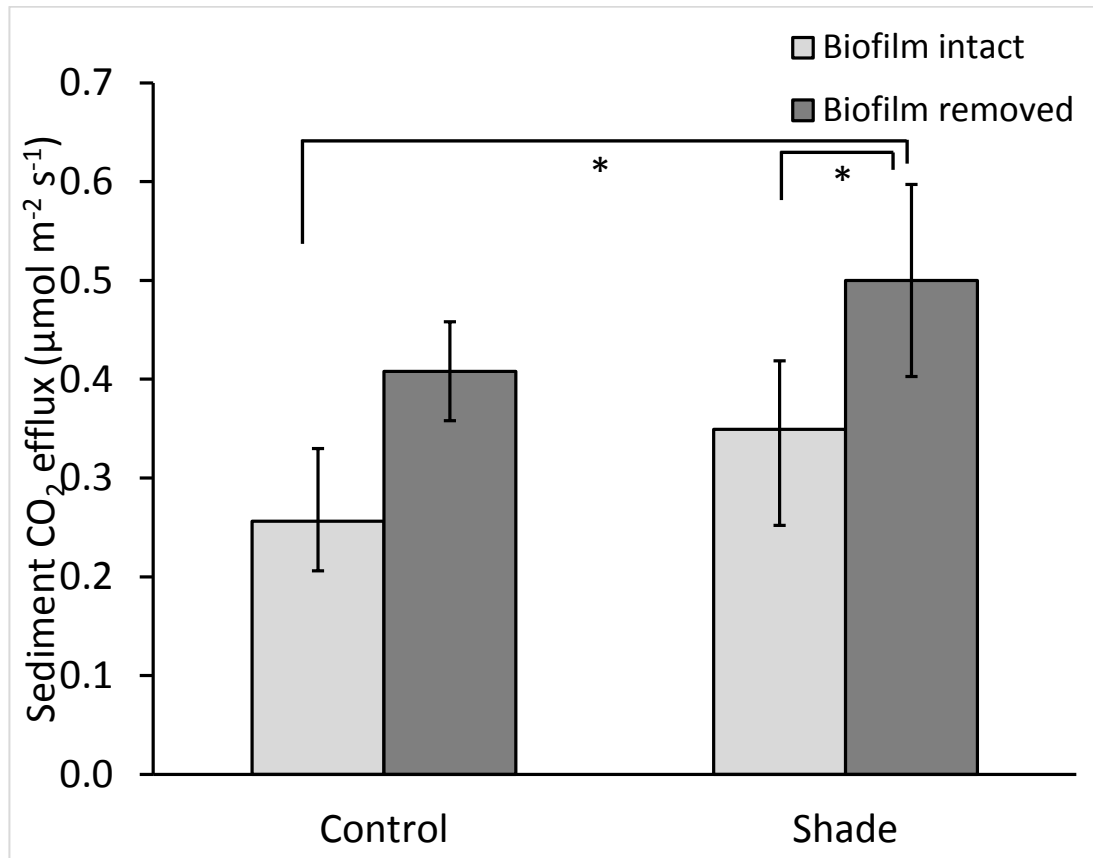


Figure 2. Mean sediment (\pm SE) CO₂ efflux ($\mu\text{mol m}^{-2} \text{s}^{-1}$) before and after surface biofilm was removed, from control ($n = 18$), and pre-shaded sediment ($n = 18$) at intact mangrove site Whangateau 2. *significant difference ($p < 0.05$)

No significant difference was detected in mean CO₂ efflux between shaded and control treatments at Whangateau 2 ($p > 0.05$). Removing the surface biofilm resulted in significantly higher CO₂ efflux for shaded treatments (Figure 2), ($p < 0.05$).

Based on these results we derive the following conclusions.

- Our procedure to measure dark CO₂ efflux (which do not include > 30 minutes of pre shading) are valid.
- Lagged photosynthetic processes within the sediment of the dark incubation chamber are unlikely to be resulting in the CO₂ uptake observed at certain sites, or the significant increase in CO₂ efflux following biofilm removal.

We have included the following in the discussion as a potential explanation of the CO₂ uptake observed at certain sites in our study.

Sediment CO₂ uptake (negative flux) was observed at one intact (Hatea 1) and three cleared (Tairua 3, Whangamata 1, Hatea 1) mangrove forest sites. CO₂ uptake has also been reported in other mangrove efflux studies (Leopold et al., 2015; Lovelock, 2008; Lovelock et al., 2014). CO₂ uptake has been explained by the presence of biofilm microbial communities, as CO₂ uptake changed to efflux following biofilm removal (Leopold et al. (2015). In other habitats, CO₂ uptake from terrestrial shrub sediment has been attributed to sediment effusion-dissolution processes driven by sediment pH and moisture (Ma et al., 2013). CO₂ uptake from wetland sediment has been attributed to the drawdown of CO₂ into the sediment during large ebbing or very low tides (Krauss and Whitbeck, 2012).

Microphytobenthos have been shown to be significant contributors to benthic primary productivity (Bouillon et al., 2008; Kristensen and Alongi, 2006; Oakes and Eyre, 2014). Due to the short duration of our measurements (90 seconds), CO₂ uptake might be explained by the continuation of photosynthetic activity by surface biofilm communities at the onset of dark measurements until coenzymes were depleted (NADPH, ATP) (Leopold et al. (2015). However, the results from our shading results suggest that this was not the case, as we did not see significantly higher CO₂ efflux from sediment that was pre-shaded compared to sediment which had not been pre shaded.

Another possibility is that the decrease in CO₂ concentration within the chamber observed at these sites is driven by the leakage of CO₂ from dark chamber measurements, via cracks, fissures or burrows in the surface sediment. The removal of the surface biofilm resulted at CO₂ emission even at the sites where CO₂ uptake was previously observed. This is possibly related to homogenising the sediment surface following biofilm removal, with cracks or burrows covered by scraped sediment, minimising CO₂ leakage to adjacent non-shaded microphytobenthos. Other studies have suggested that the biofilm may also act as a barrier to the flow of CO₂ from deeper sediment, which when removed results in a rapid increase in CO₂ efflux (Leopold et al., 2015; Leopold et al., 2013).

Chemoautotrophs have also been shown to fix carbon in intertidal sediment under dark conditions (Boschker et al., 2014; Lenk et al., 2011). In particularly at the interface of aerobic and anaerobic zones where large amounts of reduced compounds, such as sulphur, accumulate (Boschker et al., 2014; Lenk et al., 2011; Santoro et al., 2013; Thomsen and Kristensen, 1997)). This is consistent with what is observed in mangrove sediment, where aerobic to anaerobic transitions typically occur close to the sediment surface, with sulphur driven processes likely to dominate in anaerobic conditions (Kristensen et al., 2008).

Below is the response to individual referee's feedback.

Referee #1

Comment from referee: This manuscript investigates the spatial variability of CO₂ fluxes from three different intertidal systems in New Zealand: a tidal flat, an *Avicennia* mangrove stand and a cleared mangrove stand. These mangroves are the southernmost ones in the IWP area and only *Avicennia marina* can grow in this temperate climate. Opposite to what is happening in the tropics, mangroves in New Zealand are expanding mainly because of increased sedimentation as a result of increased agricultural activities in water-sheds. However, numerous clearings occurred recently notably in order to “recover recreational values of estuaries”. The main objectives of the authors were to understand the effect of mangrove clearance on sediment biogeochemistry and specifically on CO₂ fluxes from mangrove soils. To reach their goals, they measured CO₂ fluxes and collected 2-cm deep cores in numerous mangroves, cleared areas, and tidal flats at one season (late spring and summer). CO₂ fluxes were determined on the field using dark incubation chambers connected to infra-red gas analyzer before and after having removed the biofilm from sediment surface. On sediment samples, grain size, TOC, and Chl *a* content were measured. In addition, forest biomass and macrofauna distribution were determined. Methods seem to have been conducted with care and references are up to date. The main results of the authors are: i) lower CO₂ fluxes in cleared mangroves compared to *Avicennia* stand, ii) after clearance, a decrease in CO₂ fluxes with time, iii) a strong effect of biofilm on CO₂ fluxes, with increased values after biofilm removal. Mangrove forests are among the most productive terrestrial ecosystem, with high rates of carbon sequestration, both in their biomass and in their soil. Unfortunately and although there is an increasing number of studies working on it, there is still a need of data to constrain the becoming of mangrove primary productivity, notably carbon mineralization with the sedimentary column and the export of CO₂ from mangrove sediments to the atmosphere, which are underestimated and understudied, even more in temperate mangroves (e.g. see papers of Leopold et al., 2013, 2015, Lovelock et al., 2014; Chen et al., 2012, 2014). The topic is thus relevant and the references are up to date; however the ms. is characterized by flaws that do not allow its publication in its present form. Usually, I find that ms. are too long for what the authors have to say, which is the opposite with the present ms. The authors did not present enough their data, and do not discuss them enough. As a result, I believe that this paper does not have the necessary breadth and depth in terms of providing fundamental new understanding in mangrove geochemistry and ecology for a publication in Biogeosciences.

Author's response: We thank the referee for the helpful suggestions. Based on the referee's comments we modified the manuscript substantially. In particular, we re-analysed our data and re-wrote the discussion to provide an in-depth interpretation of our findings.

Comment from referee: Additionally, I have listed some points that have to be explained or modified in the ms. concerning the sampling strategy, the methods, and the presentation of their results in figures or tables. I'm not sure that it was relevant to study so many sites (40 mangrove clearance, 18 mangroves, 30 tidal flats). The authors should better describe the sites and their complementarity. With such a number of sites, the reader is expecting some figures or tables to present statistical analyses between sites, as well as for the relationships between CO₂ fluxes and the parameters that can drive them. The authors may have chosen some specific areas, where they were able to have the 3 stands together (having the same sediment characteristics, hydrology, activities in watershed, etc), and to do more analyses on these specific sites. In the same way, the authors have a lot of data, including macrofauna characteristics, but since they are not well discussed, I would suggest the authors to focus, and deeply discussed the main parameters that can explain CO₂ fluxes variability in their 3 strata. Another option would be to analyse the influence of mangrove clearings on sediment biogeochemistry and biology, not only focusing on CO₂, and to present them in a more applied journal. For instance, the authors can discuss the evolution of grain size, of the TOC content, of the macrofauna density, etc., before and after clearing.

Author's response: Based on these recommendations we have made the following modifications.

- 1. We focus on sediment CO₂ efflux and sediment characteristics from intact and cleared mangrove forest sites*
- 2. We have removed macrofaunal data from the manuscript.*
- 3. We have re-written the discussion focusing on the main factors which could influence CO₂ efflux within intact and cleared mangrove forest.*

Based on the recommendations from referee 3 we also conducted a shading experiment to investigate the impact of pre-shading the sediment. The findings of this experiment are included in the revised version of the manuscript and described above.

Comment from referee: Do the authors think that cores of 2 cm are adequate for their topic? CO₂ fluxes may be influenced by physico-chemical conditions (TOC, root respiration, redox, etc.) that are developing deeper than 2 cm. What was the limit between the saturated and the unsaturated zones at low tide during their measurements?"

Author's response: We acknowledge that deeper cores would provide a better assessment of the sediment characteristics influencing sediment CO₂ efflux. However, cores to 2 cm reflect the surface sediment conditions which are likely to be a significant driver of sediment CO₂ efflux. Additional cores were also collected to 15 cm depth and used to measure remaining root mass within cleared mangrove forest sediment. As part of a separate unpublished study conducted at four temperate mangrove sites in New Zealand, we also observed a significant positive correlation between TOC at

0-2cm and at depths from 2-4cm (r_s 0.93, $p < 0.01$), 4-6cm (r_s 0.92, $p < 0.01$), and 6-8cm (r_s 0.58, $p = 0.048$).

The limit between the saturated and unsaturated zones at low tide was not directly measured. However, tides for the sites are semi diurnal with a range of 1.3 – 4.1 m, with mangrove forest sediment typically not inundated for at least half of the tidal cycle. We have included a paragraph discussing the potential implications of measuring sediment CO₂ efflux at different time of the tidal cycle in the discussion of the manuscript.

Changes to manuscript:

We note that all sediment CO₂ efflux measurements in this study were made at low to mid-tide. The efflux of CO₂ from mangrove sediment during low tide can be up to 40% greater than during tidal immersion as molecular diffusion of CO₂ is faster when sediments are aerated and the surface area for aerobic respiration and chemical oxidation increases (Alongi, 2009). However, benthic light availability is also reduced during tidal immersion, which may result in increased respiration by the microphytobenthos (Billerbeck et al., 2007).

Comment from referee: Chla concentrations are usually highly variable at sediment surface in mangroves, thus I'm not sure that one measurement per site is enough.

Author's response: At each site three sediment samples were collected using two small sediment cores (2 cm deep, 2 cm in diameter). Chlorophyll a concentration and sediment grain size were initially measured in all three samples, however as variation between samples was small only one sample was analysed for the majority of sites. For example, sediment chlorophyll a concentration ranged from 18.92 to 22.87 $\mu\text{g}^{-1} \text{g}^{-1}$ sediment at mechanically cleared mangrove forest site Whangamata E.

Comment from referee: The authors did not measure CO₂ fluxes at light, and mentioned that their measurements exclude the uptake of CO₂ by photoautotrophic process. I agree, however they mentioned that Leopold et al did not observed any differences between light and dark measurement in Avicennia stand. I have read this paper again, and it seems that it is not directly linked to mangrove species, but rather to the position in the intertidal zone and canopy closure, that will lead to specific development of the biofilm. I do not know if the length of tidal immersion in New Zealand and canopy closure are the same that in New Caledonia for the Avicennia stands. In addition, Leopold et al. did not measure CO₂ fluxes from tidal flats, but from salt flats (so not in front of mangroves, but in the back, at higher elevation, it means different conditions of sediment oxygenation).

Author's response: We modified the discussion accordingly. Please refer to the earlier section regarding the uptake of CO₂ observed at some of our sites.

Thank you for your valuable suggestions on this manuscript.

References cited:

- Alongi, D. M.: The energetics of mangrove forests, Springer, 2009.
- Billerbeck, M., Røy, H., Bosselmann, K., and Huettel, M.: Benthic photosynthesis in submerged Wadden Sea intertidal flats, *Estuar. Coast Shelf Sci.*, 71, 704-716, 2007.
- Boschker, H. T. S., Vasquez-Cardenas, D., Bolhuis, H., Moerdijk-Poortvliet, T. W. C., and Moodley, L.: Chemoautotrophic carbon fixation rates and active bacterial communities in intertidal marine sediments, *PLoS ONE*, 9, 1-12, 2014.
- Bouillon, S., Borges, A. V., Castañeda-Moya, E., Diele, K., Dittmar, T., Duke, N. C., Kristensen, E., Lee, S. Y., Marchand, C., Middelburg, J. J., Rivera-Monroy, V. H., Smith Iii, T. J., and Twilley, R. R.: Mangrove production and carbon sinks: A revision of global budget estimates, *Global Biogeochem. Cy.*, 22, 2008.
- Krauss, K. and Whitbeck, J.: Soil greenhouse gas fluxes during wetland forest retreat along the lower Savannah river, Georgia (USA), *Wetlands*, 32, 73-81, 2012.
- Kristensen, E. and Alongi, D. M.: Control by fiddler crabs (*Uca vocans*) and plant roots (*Avicennia marina*) on carbon, iron, and sulfur biogeochemistry in mangrove sediment, *Limnol. Oceanog.*, 51, 1557-1571, 2006.
- Kristensen, E., Bouillon, S., Dittmar, T., and Marchand, C.: Organic carbon dynamics in mangrove ecosystems: A review, *Aquat. Bot.*, 89, 201-219, 2008.
- Lenk, S., Arnds, J., Zerjatke, K., Musat, N., Amann, R., and Mußmann, M.: Novel groups of Gammaproteobacteria catalyse sulfur oxidation and carbon fixation in a coastal, intertidal sediment, *Environ. Microbiol.*, 13, 758-774, 2011.
- Leopold, A., Marchand, C., Deborde, J., and Allenbach, M.: Temporal variability of CO₂ fluxes at the sediment-air interface in mangroves (New Caledonia), *Sci. Total Environ.*, 502, 617-626, 2015.
- Leopold, A., Marchand, C., Deborde, J., Chaduteau, C., and Allenbach, M.: Influence of mangrove zonation on CO₂ fluxes at the sediment-air interface (New Caledonia), *Geoderma*, 202-203, 62-70, 2013.
- Lovelock, C. E.: Soil respiration and belowground carbon allocation in mangrove forests, *Ecosystems*, 11, 342-354, 2008.
- Lovelock, C. E., Feller, I. C., Reef, R., and Ruess, R. W.: Variable effects of nutrient enrichment on soil respiration in mangrove forests, *Plant Soil*, 379, 135-148, 2014.
- Ma, J., Wang, Z.-Y., Stevenson, B. A., Zheng, X.-J., and Li, Y.: An inorganic CO₂ diffusion and dissolution process explains negative CO₂ fluxes in saline/alkaline soils, *Scientific Reports*, 3, 2025, 2013.
- Oakes, J. M. and Eyre, B. D.: Transformation and fate of microphytobenthos carbon in subtropical, intertidal sediments: potential for long-term carbon retention revealed by ¹³C-labeling, *Biogeosciences*, 11, 1927-1940, 2014.
- Santoro, A. L., Bastviken, D., Gudas, C., Tranvik, L., and Enrich-Prast, A.: Dark carbon fixation: An important process in lake sediments, *PLoS ONE*, 8, e65813, 2013.

Thomsen, U. and Kristensen, E.: Dynamics of sigma CO₂ in a surficial sandy marine sediment: the role of chemoautotrophy, *Aquat. Microb. Ecol.*, 12, 165-176, 1997.