Interactive comment on "Warming and ocean acidification may decrease estuarine dissolved organic carbon export to the ocean" by Michelle N. Simone et al.

Anonymous Referee #1 Received and published: 23 November 2020

Review of bg-2020-335 Warming and ocean acidification may decrease estuarine dissolved organic carbon export to the ocean Michelle N. Simone, Kai G. Schulz, Joanne M. Oakes, and Bradley D. Eyre

This contribution studies the effect of increased pCO_2 and temperature on the fate of DOC in photic sediments. There are two autochthonous sources for DOC in sediments: degradation of detrital POC and release from microphytobenthos. Diffusive fluxes between the overlying water and sediment pore water depend on the concentration gradient (excluding bioturbation in more permeable sediments). Increases in pCO_2 will be expected to enhance benthic primary production (and associated DOC production) while increases in temperatures will increase carbon mineralisation rates. The net effect of these combined is difficult to assess and hence the focus of this experimental study. The experiment is very well designed and carried out, and the results are clearly condensed and presented.

Comment: The results and discussion sections are, however, difficult reading, and I had to re-read many times to follow.

Reply: In addition to addressing the specific comments of both reviewers, the results and discussion section have been revised to improve clarity and readability.

Reply: Line numbers have been adjusted throughout this document to reference the revised text in response to this and the rest of the reviewer comments (see below).

Comment: I wonder if the carbon budget/fluxes can be summarised in a figure or table so it is easier for the reader to follow the net result of the treatments. I found myself doing this while reading the discussion, gathering numbers from different figures. This would great increase the impact of the paper.

Reply: Figures 2, 3 and 5 already provide a summary of flux data referred to in the main text. However, we appreciate that some readers may find it easier to refer to a table. We were happy to build a summary table of fluxes. To avoid duplication this is included as an appendix to the manuscript.

In the supplementary material you will find Table S4-S6 with data requested by Reviewer 1.

Captions read as follows:

Table S1. Gross primary productivity (GPP) and productivity to respiration ratio (P/R) calculated for each temperature under both current and high-pCO_2.

Table S2. Dark and light fluxes of dissolved organic carbon (DOC) for each temperature under both current and high-pCO_2.

Table S3. Dark and light fluxes of dissolved inorganic carbon (DIC) for each temperature under both current and high-pCO_2.

Comment: I found it misleading to always refer to the high pCO_2 scenario as ocean acidification OA. It is the increased DIC availability that is fuelling higher primary production which seems to be the major driver, rather than acidification influencing a rate as such. I recommend that this is rectified.

Reply: We agree with the reviewer, the use of OA and high-pCO_2 was simplified as a reference to high-pCO_2 only.

In the introduction we found it necessary to keep the use of OA for context, however, at the end of the introduction we have added text to highlight the distinction between OA and high-$p\text{CO}_2$. The text now reads:

LN 85: Moreover, despite the potential stimulation of primary productivity in unvegetated muddy sediments by OA (Vopel et al., 2018) or more likely high-$p\text{CO}_2$, and potential enhancement of DOC production (Engel et al., 2013; Liu et al., 2017), this increase in labile DOC may promote bacterial productivity and DOC mineralisation (Hardison et al., 2013).

Comment: It is also unclear what the nutrient levels were during the experiment. The results and discussion are focused solely on carbon limitation and assume adequate nutrient supply. That said the system the sediment cores were sampled from appears to be low nutrient. It is worth addressing this at some point.

Reply: Nutrients did not appear to be limiting in any of the treatments as nutrient concentration increased during all incubations. This is outlined in the text and the methods and data in the table below is now included in supplementary information.

In the text:

LN 353: "In comparison, nutrients were non-limiting in the less permeable sediments used in the current study, based on nutrient concentrations that increased during all incubations (see supplementary methods and Table S7)."

Supplementary methods:

Dissolved inorganic nitrogen (DIN) samples were collected at the start and end of the flux incubations and syringe-filtered (0.45 μm cellulose acetate) into duplicate 10 mL polyethylene vials with a headspace, and stored frozen. Samples were analysed colorimetrically using a Lachat™ flow-injection system as described in Eyre and Pont (2003).

Table S7. DIN concentrations (μM) (mean ± standard deviation) at the start (minimum) and end of the full incubation cycle.

Treatment	Current-$p\text{CO}_2$		High-$p\text{CO}_2$	
	Start	End	Start	End
Δ-3	1.19	2.02	1.85	6.66
	(± 0.01)	(± 0.45)	(± 0.27)	(± 1.36)
Control	1.85	4.00	2.42	6.11
	(± 0.16)	(± 0.27)	(± 1.01)	(± 1.39)
Δ+3	1.88	4.47	1.97	9.61
	(± 0.42)	(± 2.10)	(± 0.31)	(± 1.36)
Δ+5	2.37	15.52	2.40	14.68
	(± 0.18)	(± 1.81)	(± 0.58)	(± 4.42)

Comment: What effect would N limitation have on the result. Competition between MPB and heterotrophs for available nutrients for example.

Reply: This comment from Reviewer 1 addresses an important possibility in the system. We have discussed the potential effect of nutrient limitation on DOC flux in LN 412: "This failure to intercept DOC may be compounded if nutrient supply is limited (Brailsford et al., 2019), as it is common for heterotrophic bacteria to rely on refractory DOC when labile sources are not readily available (Chróst, 1991), which can occur under conditions of nutrient limited biological productivity (Allen, 1978).” (with underlined sections adjusted for clarity)

Comment: Finally, I do not see the value in scaling the data up to global estimates of sediment estuarine DOC uptake (4.3.3). It is not necessary and is fraught with very large assumptions. Similar scale ups have been done in the cited literature (Duarte papers), arrive at questionable results and conflict with current understanding of the global ocean DOC budget. The findings of this present study are relevant, intriguing and warrant publication without this final section.

Reply: We thank the reviewer for their positive comments on the relevance and interest of this study. We do, however, acknowledge the limitations of the upscaling included in the manuscript. This exercise was intended to provide a more qualitative perspective on the potential impact a future high-$p\mathrm{CO}_2$ climate could have on the DOC export from estuaries. We believe it is interesting to consider the role of unvegetated sediments in an ecosystem/global context as this system is often overlooked in carbon budgets, whereas our upscaling exercise highlights the potential importance of processes (and changes to those processes) in this environment. To address the concerns of reviewer 1, and as per Reviewer 2’s suggestion, we have added additional details of why such upscaling can be risky and possibly incorrect, including limitations such as different hydrodynamic settings, different sediment composition, different delivery of dissolved and particulate matter from land and through aeolian deposition, etc.

Comment: Specific comments Introduction **(1)** Important to distinguish between photic and aphotic sediments. They differ greatly in their role and contribution to the larger net effects of coastal waters, which are outlined at the start of the introduction. **(2)** The last part of the introduction could be rephrased to be clearer. Lines 54-78. First formulate what the dominating mechanisms acting on DOC uptake/release from photic sediments are. Then address how these mechanisms can be influenced by warmer temperatures, high CO_2, and lowered pH, respectively. Then clearly state the hypothesis you had as the basis of your experimental design.

Reply: **(1)** We agree. To clarify our focus on euphotic sediments – the restatement of this focus has been added to the final paragraph. LN 82: “We expected that warming would promote a stronger heterotrophic, than autotrophic, microbial response in shallow euphotic sediments (Patching and Rose, 1970; Vázquez-Domínguez et al., 2012; Yang et al., 2016), and as such, there would likely be more DOC remineralisation (Lønborg et al., 2018) than ‘new’ DOC production (Wohlers et al., 2009; Engel et al., 2011; Novak et al., 2018).” The focus on euphotic sediment is also now made clear in the methods, LN 95: “Sediment at the site was unvegetated and characterised as a euphotic cohesive sandy mud…” (with underlined sections adjusted for clarity)

(2) As per the reviewer’s suggestion, we have rearranged the last part of the introduction and include the recommended additions in the structure, as follows:

1 – dominating mechanisms acting on DOC:

LN 64: “Primary producers fix DIC during photosynthesis and release DOC directly through exudation and/or indirectly when they are grazed upon. Photosynthetically produced DOC is the main source of DOC in the ocean (Hansell et al., 2009). DOC fuels local microbial mineralisation (Azam, 1998). Heterotrophic bacteria respire the carbon from DOC as CO_2, which can then be recaptured by photoautotrophs (Riekenberg et al., 2018), closing the microbial loop (Azam, 1998). DOC and DIC that is not captured is ultimately effluxed to the overlying water column and may be transported from estuaries to the coastal ocean." (with underlined sections added for clarity)

2 – how warming and OA may affect these mechanisms:

LN 69: "Individually, increased temperature and CO_2 can enhance primary productivity, and therefore DOC production, in arctic (Engel et al., 2013; Czerny et al., 2013) and temperate phytoplankton communities (Wohlers et al., 2009; Engel et al., 2011; Liu et al., 2017; Novak et al., 2018; Taucher et al., 2012), and temperate stream sediments (Duan and Kaushal, 2013). However, one study in a temperate fjord reported no enhancement of DOC production despite CO_2 enhanced phytoplankton productivity (Schulz et al., 2017). This uncertainty of response to individual climate stressors is exacerbated when considering how the combination of OA and warming will affect the production and degradation of DOC. To date, only one study has considered this combined stressor effect on DOC fluxes (Sett et al., 2018), observing no difference in DOC production by temperate phytoplankton relative to current conditions (Sett et al., 2018)."

3 – Experimental design and hypotheses:

LN 77: "To understand the potential effect of future climate on DOC fluxes, it is essential that both individual and combined effects of OA and warming are considered. Here we focus on changes in DOC fluxes in unvegetated estuarine sediments, as these systems have the potential for significant uptake of DOC that is currently exported to the coastal ocean. In this study, benthic DOC responses in unvegetated estuarine sediments were investigated over an 8 °C temperature range under both current and projected future high-pCO_2 conditions in an ex situ laboratory incubation." (with underlined sections adjusted for clarity)

LN 82: "We expected that warming would promote a stronger heterotrophic, than autotrophic, microbial response in shallow euphotic sediments (Patching and Rose, 1970; Vázquez-Domínguez et al., 2012; Yang et al., 2016), and as such, more DOC remineralisation (Lønborg et al., 2018) than 'new' DOC production (Wohlers et al., 2009; Engel et al., 2011; Novak et al., 2018)." (with underlined sections adjusted for clarity)

LN 85: "Moreover, despite the potential stimulation of primary productivity in unvegetated muddy sediments by OA (Vopel et al., 2018) or more likely high-pCO_2 availability, and potential enhancement of DOC production (Engel et al., 2013; Liu et al., 2017), this increase in labile DOC may promote bacterial productivity and DOC mineralisation (Hardison et al., 2013). In addition, increased DOC availability alone may increase heterotrophic bacterial biomass production and activity (Engel et al., 2013). We therefore predicted that increases in DOC production from OA alone or in combination with warming may be counteracted by increased consumer activity, potentially diminishing the available DOC pool under future climate conditions." (with underlined sections adjusted for clarity)

Comment: What influence would variable light conditions have on your findings? The cores are taken from a shallow estuarine site where one can expect considerable resuspension from tides, currents and winds. The light intensities used here are likely representative of best case. So, one can maybe amplify the dark scenario?

Reply: This is an interesting question that would be of interest to the general readership. We see value in addressing this question within the discussion and follow the same thought process as Reviewer 1, where the dark scenario responses would likely be amplified. The following sentence was added, LN 339: "Under conditions of reduced light availability/intensity, sediments are expected to have an amplified heterotrophic response in addition to a reduction in microalgal production of DOC."

Comment: Line 7. "Estuaries make a disproportionately". What do you mean here? With respect to what?

Reply: This was unclear, the statement was adjusted to read LN 7: "Relative to their surface area, estuaries make a disproportionately large contribution of dissolved organic carbon (DOC) to the global carbon cycle, but it is unknown how this will change under a future climate." (with underlined sections adjusted for clarity)

Comment: Line 19. DOC is smaller than that retained in soils and also in fossil fuels.

Reply: While this statement by reviewer 1 is valid, we do not believe what we said is untrue, LN 20: "The aquatic dissolved organic carbon (DOC) pool is one of the largest pools of organic carbon on earth (Hedges, 1987) and roughly equivalent in size to the atmospheric CO$_2$ reservoir (Siegenthaler and Sarmiento, 1993)." We do not say it is the largest, just one of the largest. For this reason, we intend to leave this sentence unchanged.

Comment: (1) Line 28. And **(2)** line 32-35. Here you state that 33% of the NPP in coastal waters is exported to the oceans and stored in the ocean interior. I question the validity of this statement/citation**. (3)** Is there evidence that the interior ocean is increasing in DOC? Why the large difference between mineralisation efficiency of DOC produced in surface water of the ocean to that produced in coastal waters?

Reply: (1) The line reads LN 29: "up to 33 % of the associated DOC is exported offshore and stored in the ocean interior". This upper value is based on Krause-Jensen and Duarte (2016) who found that substantial macroalgal DOC produced in the coastal zone and exported offshore was subducted below the mixed layer into the ocean interior (117 (36-194) Tg-C y^{-1}). The text can be adjusted for clarity, to avoid confusion that the 33% of NPP carbon reaches the ocean interior. The text now reads, LN 28: "The shallow coastal zone accounts for 1 to 10 % of global net primary production (NPP) (Duarte and Cebrián, 1996), with up to 33 % of the associated DOC exported offshore and reaching the ocean interior (Krause-Jensen and Duarte, 2016)." (with underlined sections adjusted for clarity)

(2) There was a lack of information in this paragraph regarding how the value of 3.5× was calculated. The paragraph now reads, LN 31: "Although shallow estuaries and fringing wetlands make up only ~22 % of the world's coastal area (Costanza et al., 1997) and 8.5 % of the total marine area (Costanza et al., 1997) they are quantitatively significant in terms of DOC processing and offshore transport (Smith and Hollibaugh, 1993). In 1998, Bauer and Druffel used radioisotopic carbon (^{14}C) to identify the source and age of DOC and POC inputs into the open ocean interior. They found that ocean margins accounted for greater organic carbon inputs into the ocean interior than the surface ocean by more than an order of magnitude. Assuming 1/3 of the DOC produced in the coastal zone (100-1900 Tg-C y^{-1}, Duarte, 2017) is subducted and reaches the ocean interior (Krause-Jensen and Duarte, 2016), 30 to 630 Tg-C y^{-1}, or up to 3.5× more DOC could reach the ocean interior from coastal areas than from the open ocean (180 Tg-C y^{-1}, Hansell et al., 2009). This is despite coastal areas having a DOC production rate only 0.2 to 3.9 % that of the open ocean (Duarte, 2017). As such, small changes to the coastal production and export of DOC may have a disproportionate influence on the global DOC budget." (with underlined sections adjusted for clarity)

(3) We are not trying to suggest that the interior ocean DOC pool is increasing, but instead, that a disproportionally large amount of DOC in the interior ocean could be sourced from the coastal zone relative to the surface ocean. This is based on previous work looking into the transport of DOC from the coastal zone and surface ocean to the ocean interior, respectively (calculations detailed in (2)).

We have included in our introduction the following text to further support the importance and potential significance of changing the supply of coastal DOC to the ocean.

LN 33: "In 1998, Bauer and Druffel used radioisotopic carbon (^{14}C) to identify the source and age of DOC and POC inputs into the open ocean interior. They found that ocean margins accounted for greater organic carbon inputs into the ocean interior than the surface ocean by more than an order of magnitude."

Comment: Line 43. Delete extra "lability"

Reply: Thank you. This has been rewritten to avoid repeating "lability". LN 47: "These heterotrophic bacteria not only consume autochthonous DOC (Boto et al., 1989), but their biomass is influenced by the lability of sediment organic matter (OM) (Hardison et al., 2013), which can be directly linked to and stimulated by MPB (Hardison et al., 2013; Cook et al., 2007)." (with underlined sections adjusted for clarity)

Comment: First three paragraphs contradict. You start by arguing that coastal waters are an important source of DOC to the open ocean but then finish by stating that coastal sediments are an important sink for DOC.

Reply: This can be clarified by exaggerating the distinction between coastal zone as a whole and estuarine sediments as a part of that whole in the third paragraph. The intention is to highlight that the coastal zone is an important source of DOC for the global ocean, however in sediments heterotrophic bacteria can make unvegetated estuarine sediments a sink of DOC produced elsewhere. As such, it is important to assess the role of this potential sink under conditions of warming and OA. The third paragraph has therefore been adjusted below:

LN 41: "Euphotic estuarine sediments occupy the coastal boundary between terrestrial and marine ecosystems. Microalgal communities (microphytobenthos, or MPB) are ubiquitous in these sediments, occupying ~40 to 48 % of the coastal surface area (Gattuso et al., 2020), and generating up to 50 % of total estuarine primary productivity (Heip et al., 1995; MacIntyre et al., 1996; Underwood and Kromkamp, 1999). MPB exude some of the carbon they fix as extracellular substances, including carbohydrates (Oakes et al. 2010), and can therefore be a source of relatively labile DOC in net autotrophic sediments (Cook et al., 2004; Oakes and Eyre, 2014; Maher and Eyre, 2010). However, microbial mineralisation by heterotrophic bacteria (Azam, 1998) within the sediment communities are a dominant sink of DOC in coastal sediments (Boto et al., 1989). These heterotrophic bacteria not only consume autochthonous DOC from upstream (Boto et al., 1989), but their biomass is influenced by the lability of sediment organic matter (OM) (Hardison et al., 2013), which can be directly linked to and stimulated by MPB (Hardison et al., 2013; Cook et al., 2007). As such, estuarine sediments are a potentially important sink for DOC." (with underlined sections adjusted for clarity)

Comment: Line 48. Check referencing. Fischot and Benner paper does not address the processing of DOC by estuarine sediments.

Reply: This is true. Fichot and Benner (2014) looks at shelf processes, not estuarine. However, it is likely that the euphotic unvegetated shelf sediments in Fichot and Benner (2014) would not be dissimilar to euphotic unvegetated estuarine sediments. A more nearshore reference would be by Sandberg et al. (2004), who found that tDOC was the dominant carbon source for bacterial secondary production in the water column of Ore Estuary (Northern Baltic Sea).

This has been reworded in the text as follows:

LN 51: **“**Unvegetated estuarine sediments can affect the quantity and quality of DOC input to the ocean by 1) acting as a source of autochthonous DOC, through MPB production (Duarte, 2017; Krause-Jensen and Duarte, 2016; Maher and Eyre, 2010), or 2) modifying allochthonous and terrigenous DOC inputs (Fichot and Benner, 2014). Through efficient mineralisation of DOC (Opsahl and Benner, 1997), estuaries can act as a sink for DOC and a source of CO_2 to the ocean (Frankignoulle et al., 1998; Fichot and Benner, 2014; Sandberg et al., 2004).” (with underlined sections adjusted for clarity)

Comment: Line 55-60. The increased DOC production in the Engel et al 2013 study was due to nutrient limitation. When they added nutrients, it was rapidly removed again. So, no net accumulation of DOC.

Reply: This reference was removed from this section.

Comment: Line 287-289. This can be deleted.

Reply: Agreed, it was deleted.

Comment: Line 340-343. Check phrasing and possible break into two sentences to make easier reading.

Reply: The sentence has been adjusted for clarity. LN 350: “As well as differences in diffusive versus advective modes of solute transfer between the sediment types (Cook and Røy, 2006), differences may be partially due to sandier sediments being limited by other factors such as nutrient and OM availability, given that coarser sediments are generally more oligotrophic (Admiraal, 1984; Heip et al., 1995).” (with underlined sections adjusted for clarity)

Comment: Line 350-359. Here the authors begin to speculate about the lability of DOC without any measurements to support it. I am not sure it is necessary.

Reply: We see what Reviewer 1 is saying. This paragraph functions without that sentence. As such, it was deleted.

Comment: Line 395. DOC is also produced continually from the detrital sediment POC. This contributes to dark DOC production.

Reply: We have added this source of dark DOC in the discussion.

LN 408: “Although DOC is mainly produced by photoautotrophs, DOC can be produced in the dark through, for example, chemodegradation of detrital organic carbon and cell lysis by viruses and during grazing (Carlson, 2002). ”

Comment: Line 398-399. Are you inferring nutrient limitation in your set up? For now, I have assumed you had adequate nutrients.

Reply: There were no apparent N limitations in the present study, however, we were opening up the discussion to gauge what could happen if there was a limitation in nutrients. The responses to Reviewer 1's comments, detailed above, add extra clarity to the nutrient availability for the sediments.

Comment: Line 401. A very bold statement and the reference (Costanza) does not seem to support it. Please check.

Reply: The reference was incorrect. Explanation for how this was calculated is now provided in the introduction LN 35: "Assuming 1/3 of the DOC produced in the coastal zone (100-1900 Tg-C y^{-1}, Duarte, 2017) reaches the ocean interior (Krause-Jensen and Duarte, 2016), 30 to 630 Tg-C y^{-1}, or up to 3.5× more DOC could reach the ocean interior from coastal areas than from the open ocean (180 Tg-C y^{-1}, Hansell et al., 2009)." and this reference now reads, LN 417: "Up to 3.5× more DOC reaches the ocean interior from coastal areas than the open ocean (Duarte, 2017; Krause-Jensen and Duarte, 2016; Hansell et al., 2009).

Comment: **(1)** Figures Error bars in the figure should go both plus and minus. **(2)** Check text in figure 4. Do you not mean aerobic respiration (with arrow pointing upwards)?

Reply: **(1)** The figures were changed into box and whisker plots to show the full range of data. This should satisfy Reviewer 1 and 2's concerns.

(2) We thank Reviewer 1 for catching this oversight. The arrows that are now on the figure are accurate.

Anonymous Referee #2 Received and published: 27 November 2020

This is a well described experimental case study that contributes to close an important knowledge gap concerning the modification of the carbon cycle under global environmental and climatic change. My biggest concern in the study is the upscaling to the global dimension. The authors are aware of the associated risks and that such an upscaling may be (at least) quantitatively quite problematic. Overall, this is a thoroughly made study and a useful addition in the field.

Suggestions for a revised manuscript:

Comment: Section 4.3.3: The authors are correct in being very careful when they provide a daring global upscaling here. It would be good to add a paragraph on detailing why such an upscaling can be risky and possibly incorrect (different hydrodynamic settings, different sediment composition, different delivery of dissolved and particulate matter from land and through aeolian deposition, etc.)

Reply: We agree. This section is highly speculative and is purely an exercise of interest, a likely exercise that readers will do on their own. We had follow Reviewer 2's suggestion and add further details regarding the limitations of the upscaling. Also, see our reply to Reviewer 1's comments.

Comment: Line 54: It is not only the climate project models but rather the scenarios used for the projections. The scenarios are usually produced through simplified climate models and integrated assessment models.

Reply: Yes, this is true. We had included the scenario reference at the end of the sentence (RCP8.5), however, it would be more forthcoming to include the "high-emission scenario climate projections" explicitly in the text. This adjustment has been added.

LN 59: “Climate projection models assuming a high-emission scenario suggest that atmospheric CO_2 concentrations could more than double by the end of the century, increasing the partial pressure of CO_2 ($p$$CO_2$) in surface waters to 1000 μatm and decreasing pH by 0.3 units, together termed ocean acidification (OA) (RCP8.5, IPCC, 2019).”

Comment: Line 55: “increasing the partial pressure by 580 ppm” – relative to which reference year?

Reply: This has been rewritten for clarity. LN 59: “CO_2 concentrations could more than double by the end of the century, increasing the partial pressure of CO_2 ($p$$CO_2$) in surface waters to 1000 μatm …”

Comment: Lines 55-60: Though regional primary production may be enhanced with temperature and $p$$CO_2$, climate change can lead to increased stratification and a decrease of mixing as well. It would be good to also discuss this aspect and cite a few relevant literature sources.

Reply: This discussion of the possible effect of stratification was be added to the discussion section with the following text: LN 428:“ For example, the response to warming and $p$$CO_2$ may be different for pelagic communities and/or in deeper waters that are subject to stratification (Li et al., 2020), where access to nutrients and CO_2 may become limiting (Rost et al., 2008).”

Comment: Line 140: “refit from Mehrbach et al. (1973)” – can you describe in more detail how and why you did this?

Reply: We did not do the refit, Dickson and Millero (1987) did. The sentence reads, “Total borate concentrations (Uppström, 1974) and boric acid (Dickson, 1990) and stoichiometric equilibrium constants for carbonic acid (Dickson and Millero, 1987), refit from Mehrbach et al. (1973), were used.” We just wanted to include the original source of Dickson and Millero (1987). For clarity, this has been rewritten as LN 151: “…carbonic acid from Mehrbach et al. (1973) as refit by Dickson and Millero (1987), were used.”

Comment: Line 277: “OA alone (at ambient temperatures)” – what is meant with ‘ambient temperatures’ exactly?

Reply: At ambient temperatures was meant to distinguish the OA scenario from the OA and temperature manipulation scenarios. This would therefore be at 23 °C. This sentence would be improved with the addition of the temperature included. The text now reads, LN 220: “High-$p$$CO_2$ alone (at mean ambient temperatures, 23 °C)”

Comment: Section headings “4.2 OA increases DOC uptake” and “4.3.2 Warming increases respiration and DOC uptake” are unclear. Which component takes up DOC? Maybe use a different word for ‘uptake’?

Reply: We can see the ambiguity in uptake. We believe assimilation would be a more accurate term as the heterotrophs in the sediments actively assimilate DOC. The section headings now read: LN 341: “4.2 OA increases DOC assimilation” and LN 367: “4.3 Warming drives increased heterotrophy and DOC assimilation” and LN 395: “4.3.2 Warming increases respiration and DOC assimilation”

Comment: Figure 1: Some fonts are so tiny that they are not readable. Please, increase them if relevant or delete unnecessary information.

Reply: This was adjusted as suggested.

Comment: Figure 5: The ‘bars’ within the grey and dotted areas of the plot are barely visible. What do these ‘bars’ show? Please, provide information in the figure caption.

Reply: The figure has been redesigned. The figure caption now clearly indicate "Light (grey boxes) and dark fluxes (spotted boxes) of DOC (μmol-C m^{-2} h^{-1}) for (b) current-pCO$_2$ and (c) high-pCO$_2$ conditions."

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Warming and ocean acidification may decrease estuarine dissolved organic carbon export to the ocean

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Abstract. ~~Estuaries~~Relative to their surface area, estuaries make a disproportionately large contribution of dissolved organic carbon (DOC) to the global carbon cycle, but it is unknown how this will change under a future climate. As such, the response of DOC fluxes from microbially dominated unvegetated sediments to individual and combined future climate stressors of warming (from Δ-3 °C to Δ+5 °C ~~on~~compared to ambient mean temperatures) and ocean acidification (OA, ~2~~ times the~~× current CO_2 partial pressure~~ of CO_2~~, $p\mathrm{CO_2}$) was investigated ex situ. Warming alone increased sediment heterotrophy, resulting in a proportional increase in sediment DOC uptake~~, with~~; sediments ~~becoming~~became net sinks of DOC (3.5 to 8.8 mmol-C m^{-2} d^{-1}) at warmer temperatures (Δ+3 °C and Δ+5 °C, respectively). This temperature response changed under OA conditions, with sediments becoming more autotrophic and a greater sink of DOC (~~1~~up to 4~~ times~~× greater than under current-$p\mathrm{CO_2}$). This response was attributed to the stimulation of heterotrophic bacteria with the autochthonous production of labile organic matter by microphytobenthos. Extrapolating these results to the global area of unvegetated subtidal estuarine sediments, the future climate of warming (Δ+3 °C) and OA may decrease ~~the~~ estuarine export of DOC by ~80 % (~150 Tg-C yr^{-1}) and have a disproportionately large impact on the global DOC budget.

1 Introduction

The aquatic dissolved organic carbon (DOC) pool is one of the largest pools of organic carbon on earth ~~-~~(Hedges, 1987)~~ and~~, roughly equivalent in size to the atmospheric CO_2 reservoir (Siegenthaler and Sarmiento, 1993). The role of DOC in ~~the~~ long-term carbon storage~~ of carbon~~ in the ocean has been a focus of research for decades (Siegenthaler and Sarmiento, 1993; Hansell et al., 2009; Bauer and Bianchi, 2011; Wagner et al., 2020), with DOC reaching the ocean interior being effectively stored for millennia (Hansell et al., 2009). Although phytoplankton in the surface ocean are the main source of DOC globally, with an estimated production of around 50 Pg-C yr^{-1}, only 0.3 % of the DOC ~~produced by phytoplankton~~they produce reaches the ocean interior (Hansell et al., 2009)~~, with most~~. Most of the DOC produced by phytoplankton is rapidly remineralised in the water column by heterotrophic bacteria~~ in the water column~~ (Azam, 1998). Only more recently has the coastal zone been considered a major source of DOC export to the open ocean and deep-sea (Duarte et al., 2005; Maher and Eyre, 2010; Krause-Jensen and Duarte, 2016). The shallow coastal zone accounts for 1 to 10 % of global net primary production (NPP) (Duarte and Cebrián, 1996), ~~and~~with up to 33 % of the associated DOC~~ is~~ exported offshore and stored in the ocean interior (Krause-Jensen and Duarte, 2016).

Although shallow estuaries and fringing wetlands make up only ~22 % of the world's coastal area (Costanza et al., 1997) and 8.5 % of the total marine area (Costanza et al., 1997) they are quantitatively significant in terms of DOC processing and offshore transport (Smith and Hollibaugh, 1993). ~~The quantity of DOC reaching the ocean interior from coastal areas is up to 3.5 times more than that derived from production in the surface ocean.~~In 1998, Bauer and Druffel used radioisotopic carbon (^{14}C) to identify the source and age of DOC and POC inputs into the open ocean interior. They found that ocean margins accounted for greater organic carbon inputs into the ocean interior than the surface ocean by more than an order of magnitude. Assuming 1/3 of the DOC produced in the coastal zone (100-1900 Tg-C y^{-1}, Duarte, 2017) is subducted and reaches the ocean interior (Krause-Jensen and Duarte, 2016), 30 to 630 Tg-C y^{-1}, or up to 3.5× more DOC could reach the ocean interior from coastal areas than from the open ocean (180 Tg-C y^{-1}, Hansell et al., 2009). This is despite coastal areas having a DOC production rate only 0.2 to 3.9 % that of the open ocean (Duarte, 2017). As such, small changes to the coastal production and export of DOC may have a disproportionate influence on the global DOC budget.

Euphotic estuarine sediments occupy the coastal boundary between terrestrial and marine ecosystems. Microalgal communities (microphytobenthos, or MPB) are ubiquitous in these sediments, occupying ~40 to 48 % of the coastal surface area (Gattuso et al., 2020), and generating up to 50 % of total estuarine primary productivity (Heip et al., 1995; MacIntyre et al., 1996; Underwood and Kromkamp, 1999). MPB exude some of the carbon they fix as extracellular substances, including carbohydrates (Oakes et al. 2010), and can therefore be a source of relatively labile DOC in net autotrophic sediments (Cook et al., 2004; Oakes and Eyre, 2014; Maher and Eyre, 2010). The dominant sink of DOC in estuarine sediments, however, is ~~microbial mineralisation~~uptake by heterotrophic bacteria (Azam, 1998). These heterotrophic bacteria not only consume autochthonous DOC from upstream (Boto et al., 1989), but their biomass is influenced by the lability of sediment organic matter (OM) ~~lability~~ (Hardison et al., 2013), which can be ~~directly linked to and stimulated by the MPB productivity of the sediments~~altered by MPB production (Hardison et al., 2013; Cook et al., 2007). Estuarine sediments are therefore a potentially important sink for DOC.

~~Although~~Unvegetated estuarine sediments can ~~be~~affect the quantity and quality of DOC input to the ocean by 1) acting as a source of autochthonous DOC ~~to the ocean~~, through MPB production (Duarte, 2017; Krause-Jensen and Duarte, 2016; Maher and Eyre, 2010), ~~they also control the quantity and quality of~~or 2) modifying allochthonous and terrigenous DOC ~~(tDOC) that passes through them~~inputs (Fichot and Benner, 2014). ~~tDOC supports heterotrophy in estuaries and unvegetated coastal zones, which are often sources~~Through efficient mineralisation of DOC (Opsahl and Benner, 1997), estuaries can act as a sink for DOC and a source of CO$_2$ to the ocean (Frankignoulle et al., 1998; Fichot and Benner, 2014; Sandberg et al., 2004)~~. Much of the tDOC is efficiently mineralised in estuaries before it reaches the open ocean (Opsahl and Benner, 1997). The~~. Given the disproportionate contribution of estuaries to the export of DOC to offshore marine ecosystems, relative to their surface area ~~requires a better understanding of~~, it is important to understand how ~~the~~this balance of DOC sources and sinks within estuaries may change ~~in the~~with future ~~when exposed to a high-CO$_2$~~ shifts in climate ~~of increased temperatures~~, particularly expected increases in temperature and ocean acidification (OA~~).~~) associated with elevated atmospheric CO$_2$ concentrations.

Climate projection models assuming a high-emission scenario suggest that atmospheric CO_2 concentrations could more than double by the end of the century, increasing the partial pressure of CO_2 (pCO_2 ~~= +580 μatm), decreasing pH (~0.3 units, OA), and increasing temperature (Δ+2 to 4 °C) in the surface ocean (RCP8.5; IPCC, 2013).~~) in surface waters to 1000 μatm and decreasing pH by 0.3 units, together termed ocean acidification (OA) (RCP8.5, IPCC, 2019). There is also expected to be an increase in mean surface ocean temperature by 2-4°C (RCP8.5, IPCC, 2019) and increased frequency of unseasonably warm days (Morak et al., 2013; Fischer and Knutti, 2015).

Primary producers fix DIC during photosynthesis and release DOC directly through exudation and/or indirectly when they are grazed upon. Photosynthetically produced DOC is the main source of DOC in the ocean (Hansell et al., 2009) and fuels local microbial mineralisation (Azam, 1998). Heterotrophic bacteria within estuarine sediments respire the carbon from DOC as CO_2, which can then be recaptured by photoautotrophs (Riekenberg et al., 2018), closing the microbial loop (Azam, 1998). DOC and DIC that is not captured is ultimately effluxed to the overlying water column and may be transported from estuaries to the coastal ocean. Individually, increased temperature and CO_2 have been reported to enhance primary productivity and DOC production in arctic ~~(Engel et al., 2013; Czerny et al., 2013)~~(Czerny et al., 2013) and temperate phytoplankton communities (Wohlers et al., 2009; Engel et al., 2011; Liu et al., 2017; Novak et al., 2018; Taucher et al., 2012), and temperate stream sediments (Duan and Kaushal, 2013). However, one study in a temperate fjord reported no enhancement of DOC production despite CO_2 enhanced phytoplankton productivity (Schulz et al., 2017). This uncertainty of response to individual climate stressors is exacerbated when considering how the combination of OA and warming will affect DOC processing. To date, only one study has considered this combined stressor effect on DOC (Sett et al., 2018), observing no difference in DOC production by temperate phytoplankton relative to current conditions under the combined stressors (Sett et al., 2018).

To understand the potential effect of future climate on DOC fluxes ~~under future climate conditions~~, it is essential that ~~we consider~~ both individual and combined effects of OA and warming~~.~~ are considered. Here we focus on ~~the~~ changes in DOC fluxes in unvegetated estuarine sediments, as these systems have the potential ~~to take up~~for significant ~~portions~~uptake of DOC that is currently exported to the coastal ocean. In this study, benthic DOC responses in unvegetated estuarine sediments were investigated over an 8 °C temperature range under both current and projected future high-pCO_2 conditions in an ex situ laboratory incubation.

We expected that warming would promote a stronger heterotrophic, than autotrophic, microbial response in shallow euphotic sediments (Patching and Rose, 1970; Vázquez-Domínguez et al., 2012; Yang et al., 2016), and as such, there would likely be more DOC remineralisation (Lønborg et al., 2018) than ‘new’ DOC production by photoautotrophs (Wohlers et al., 2009; Engel et al., 2011; Novak et al., 2018). Moreover, despite the potential stimulation ~~by OA~~ of primary productivity in unvegetated muddy sediments by OA (Vopel et al., 2018), or more likely high-pCO_2, and potential enhancement of DOC production (Engel et al., 2013; Liu et al., 2017), this increase in labile DOC may promote bacterial productivity and DOC mineralisation (Hardison et al., 2013). In addition, increased DOC availability alone may increase heterotrophic bacterial biomass production and activity (Engel et al., 2013). We therefore predicted that increases in DOC production from OA alone or in combination with warming may be counteracted by increased consumer activity, ~~depleting~~potentially diminishing the available DOC pool under future climate conditions.

2 Methods

2.1 Study site

A subtidal site (~1.5 m below mean sea level) in the subtropical Clarence River Estuary, Australia, was used for this study (29°24.21’S, 153°19.44’E; Figure 1). Sediment at the site was unvegetated and characterised as a euphotic cohesive sandy mud (31-36 % grains 250-500 μm, 61-65 % 63-250 μm, and ~2% <63 μm, Lewis and McConchie, 1994). Temperature ± 0.3 °C, pH ± 0.5 units, and salinity (± <1 %) were measured over 24 hours using a Hydrolab (HL7) submerged at the site. The tidal cycle introduced a salinity range of 10-35, pH range of 7.~~42~~92-8.15 units (min-max), and mean daily temperature of 23.9 ± 1.6 °C (20-25 °C). The surface sediments (0-2 cm) had a porosity of 0.43 and an organic matter content of ~3.5 % (of dry weight), determined from mass loss after combustion (490 °C) of dried sediment (60 °C) (Luczak et al., 1997). The Clarence River Estuary has low nutrient loading (Eyre and Pont, 2003) with dissolved inorganic nitrogen (DIN) concentrations <2 μM (Eyre, 2000). This is consistent with concentrations determined at the time of this study (~0.9-1.9 μM DIN, Chapter 4).

2.2 Core collection

Sediment (~20 cm depth) was collected and capped in acrylic cores (9 cm diameter x 47 cm length) allowing for ~1.8 L of overlying water on the 9th (~~33~~15 cores) and 16th (~~27~~12 cores) of January 2018. Thalassinidean shrimp, *Trypaea australiensis*, burrows were avoided and therefore excluded from the collected cores as their occasional inclusion would result in considerable variability in sediment processes (Webb and Eyre, 2004) that would mask potential treatment effects. To ensure sediments were subtidal, cores were collected during low tide. Immediately after core collection, ~700 L of site water was also collected to fill a laboratory incubation setup.

2.3 Incubation setup

Within 6 hours of core collection, all cores were in the laboratory, submerged, uncapped in site water. The cores were placed in 1 of 4 temperature tanks, Control (23 °C), Δ-3 °C (20 °C), Δ+3 °C (25 °C), and Δ+5 °C (28 °C) filled with ~80 L of site water, with temperatures maintained and monitored via thermo-regulating aquarium pumps. Each tank had ~~two sets of~~ 3 cores (n = ~~6) except for the Control tank, which had an additional~~ 3 ~~cores (n = 9) for background isotope determination.~~).

The ex situ study design allowed control of temperature, pCO_2 and light that would be difficult to achieve in situ. Due to limited space, this investigation was conducted over two weeks with two complementary incubations repeated back-to-back. The incubation in the first week (January 9-12, 2018) had cores in the 4 temperature tanks subjected to ~~future~~high-pCO_2 (~1000 μatm), achieved with a CO_2 enriched airstream (initially adjusted and set when attached to a LICOR (LI-7000)) bubbled into tank water via airstones and air pumps~~ to simulate the future atmospheric CO_2 condition (~1000 µatm; RCP8.5, Collins et al., 2013), whereas the~~. The incubation in the second week (January 16-20, 2018) maintained current-pCO_2 (~450 μatm) by circulating ambient laboratory air through the tank water via airstones and air pumps. An additional tank was included in week one alongside the ~~future~~high-pCO$_2$ incubation. This tank was a control tank equivalent to the control tank present ~~in the current-~~ ~~pCO$_2$ incubation~~week two, allowing for comparison of the two separate incubations (see Table 1 for details). The temperature and pCO$_2$ manipulations were within 12 % and 4%, respectively, of their in situ ranges (see Sect. 2.1) to reduce any potential shock effect for the sediment community.

Water columns within cores were stirred at ~60 rpm throughout the incubations via magnetic stir bars (~5 cm above sediment surface) interacting with an external rotating magnet, ensuring water columns were well mixed whilst avoiding sediment disturbance (Ferguson et al., 2003, 2004). High pressure sodium lamps (400 W; PHILIPS Son-T Argo 400) were used to simulate mean daytime field conditions, providing ~270-280 μmol quanta m^{-2} s^{-1} of photosynthetically active radiation (PAR) at the water surface of the tanks. Lamps were turned on in the mornings in line with natural diel light cycling, following a similar in situ ~12:12 hour dark:light cycle. ~~After 24-48 hour of cores preincubating~~Cores were pre-incubated at treatment conditions~~, cores were capped~~ for ~~a short-term 20 hour (10:10 hour, dark:light)~~36-48 hours, before solute-flux incubations. This pre-incubation ~~to measure rates of O$_2$, DIC, and DOC production and consumption over a diel cycle. The temperature manipulations remained within 12 % of their in situ ranges (see Sect. 2.1) to reduce any potential shock effect that may be experienced by the sediment community in a short-term incubation. The two day pre-incubation~~period would be sufficient for ~~up to~~ three or six generations of the dominant microbial members of unvegetated estuarine sediments, diatoms and cyanobacteria, respectively (Mori et al., 1996; Greene et al., 1992), allowing time for the microbial community to acclimatise to the new treatment conditions.

2.3.1 Solute flux incubation

~~2.3.1~~1.1.1 Immediately after pre-incubation, cores were capped for a 20 hour (10:10 hour, dark:light) solute-flux incubation to measure rates of O$_2$, DIC, and DOC production and consumption over a diel cycle. ~~Solute flux incubation~~

~~Carbon fluxes were measured over a 20 hour period from three cores from each tank.~~ To adhere to natural diel cycling, cores were capped at dusk to start the incubation on a dark cycle. Samples were collected ~~at~~from three cores per tank at each of three time points in the diel cycle (dark start (dusk), dark end/light start (dawn), and light end (dusk)). Water was collected and syringe-filtered to determine concentrations of DIC (0.45 μm Minisart filter, 100 ml serum bottle; without headspace, poisoned with 50 μl of saturated HgCl$_2$, stored at room temperature) and DOC (GF/F filter, 40 ml glass vial with silicon septum; without headspace, poisoned with 20 μl of HgCl$_2$, injected with 200 μl of 85 % H$_3$PO$_4$, stored at room temperature). As water was removed for sampling it was replaced with gravity-fed water maintained in a collapsible bag under the same atmospheric conditions and temperature. After all cores were sampled, dissolved oxygen (DO) concentrations, temperature, and pH were measured using a high precision Hach HQ40d Multiprobe meter with an LDO-probe and pH-probe, calibrated to 3-point NIST buffer scale (R^2 = 0.99). Probes were inserted into a resealable port fitted in each lid, ensuring no incubation water exchanged with tank water. After the dawn sampling (time point 3), lamps were switched on.

DIC concentrations were determined with an AIRICA system (MARIANDA, Kiel) via infrared absorption using a LI-COR LI-7000, and corrected for accuracy against certified reference material, batch #171 (Dickson, 2010). Measurements on four analytical replicates of 1.5 ml sample volume were used to calculate DIC concentration as the mean of the last three out of four measurements (typical overall uncertainty, <1.5 μmol kg^{-1}). DIC and pH measurements were then used to calculate the remaining carbonate chemistry parameters (Table 1) using ~~CO_2Sys~~CO_2SYS (Pierrot et al., 2006). Total borate concentrations (Uppström, 1974) and boric acid (Dickson, 1990) and stoichiometric equilibrium constants for carbonic acid ~~(Dickson and Millero, 1987), refit~~ from Mehrbach et al. (1973), as refit by Dickson and Millero (1987), were used. ~~A comparison~~Comparison of ~~measured~~ pH (free scale) measured with a Hach HQ40d Multiprobe meter and pH calculated ~~pH using~~from measured total alkalinity and DIC (Table S4~~),~~) indicated an uncertainty ~~of ± 0.05 pH units~~ for potentiometric pH measurements ~~without synthetic seawater buffers. Assuming the same~~of ± 0.05 pH units. Propagating the uncertainty in pH measurements ~~in this study and propagating it~~ with the uncertainty of DIC~~, this~~ measurements, translates to a pCO_2 uncertainty of ± ~110 and ~56 μatm under ~~future~~high and current-pCO_2, respectively. This uncertainty is well within the treatment variability measured among cores (Table 1) and is therefore considered unlikely to have contributed substantially to differences in treatment response. DOC concentrations were measured via continuous-flow wet-oxidation using an Aurora 1030W total organic carbon analyser (Oakes et al., 2011) (uncertainty of ~3 %).

2.4 Data analysis

The dissolved oxygen and DIC measurements were used to estimate benthic microalgal production inside the cores. Net primary production and respiration (NPP and R, μmol-O_2 m^{-2} h^{-1}) were defined as the light or dark cycle oxygen evolution, respectively, where DIC and DOC light and dark fluxes (μmol-C m^{-2} h^{-1}) were defined using the evolution of DIC and DOC concentrations, respectively. ~~Flux~~Fluxes (NPP, R, DIC, or DOC) ~~was~~were calculated as:

$$\text{Flux} = \frac{(\text{End} - \text{Start}) \times V}{(T \times A)} \qquad \text{Eq. (1)}$$

where End and Start are the dissolved oxygen, inorganic carbon, or organic carbon concentrations (μmol-O_2 or -C L^{-1}) at the end and start of the light or dark cycle, V is the water column volume (L), T is hours of incubation, and A is surface area of the core.

Gross primary productivity (GPP, μmol-O_2 m^{-2} h^{-1}) was calculated using NPP~~/R.~~ and R, as follows:

$$\text{GPP} = -\text{R} + \text{NPP} \qquad \text{Eq. (2)}$$

The production to respiration ratio (P/R) was calculated using GPP and R~~ rates~~ scaled for a 12:12 hour light:dark diel cycle (Eyre et al., 2011).

$$\text{P/R} = \cancel{\frac{(\text{GPP}) \times 12\text{hr}}{(-\text{R} \times 24\text{hr})}}\frac{(\text{GPP}) \times 12\text{h}}{(-\text{R} \times 24\text{h})} \qquad \text{Eq. (3)}$$

Finally, net fluxes for DIC and DOC were calculated from the dark and light fluxes from Eq. (1) and presented as mmol-C ~~per m^2 per day~~m^{-2} d^{-1} for a 12:12 hour light:dark diel cycle.

$$\text{Net flux} = ((\text{Dark flux x 12h}) + (\text{Light flux x 12h}))/1000 \qquad \text{Eq. (4)}$$

Temperature sensitivity coefficients (Q_{10} values) were used to evaluate the temperature dependence of metabolic rates to temperature increases of 10 °C. This was expressed simply as an exponential function:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{10°C/(T_{opt}-T_1)} \qquad \text{Eq. (5)}$$

where R_1 and R_2 are the R, NPP, or GPP rates measured at temperatures 20 °C (T_1) and optimal temperatures (T_{opt}), where rates are highest, respectively.

2.4.1 Scaling rates

Rates in the overlapping control cores each week were checked to ensure comparability between incubations. If means (± SD) were significantly different (did not overlap), rates from individual treatment cores were scaled to the overall mean control rate of both weeks (n = 6). This was done by calculating the relative proportion of treatment rates (tProp., μmol-N m^{-2} h^{-1}) to the control rates present in its week (~~n = 3),~~Eq. (6)),

$$\text{tProp.} = \frac{\text{tRate}}{\text{Control}} \qquad \text{Eq. (6)}$$

where tRate is the individual core rate, and ~~tProp.~~Control is the ~~proportional core~~ mean rate ~~from the mean~~ of control ~~(Control, n = 3)~~cores present ~~in its week.~~during the incubation (n = 3). This proportional rate was then multiplied by the overall control mean rates (averaged across both weeks, n = 6) to scale individual core rates and calculate comparable treatment means (n = 3) across incubations (see Sect. 3.1 for details on scaled rates).

2.5 Statistical analysis

Homogeneity of variances (Levene's test) were tested before analysis to minimize the potential for type I error ~~potential~~. All tests were run in MATLAB (Mathworks, 2011) with significance defined at a maximum alpha of < 0.05. Where Levene's test returned a significant result, datasets were either log transformed or, if negative values were present, an alpha of 0.01 was used for the ~~following~~subsequent ANOVAs.

2.5.1 Net variability with temperature and CO$_2$

Net fluxes were compared among treatments ~~were compared~~ to identify the individual and combined effects of temperature and pCO$_2$ on O$_2$, DIC, and DOC fluxes. To investigate the effect of increased pCO$_2$ alone, data from control temperature cores at both current and ~~future~~high-pCO$_2$ (n = 2) were compared using a ~~paired-sample t-test. One~~one-way analyses of variance (ANOVA). A two-way ANOVA on each dataset identified whether there were interacting effects on O$_2$, DIC and DOC fluxes of temperature (n = 4) and pCO$_2$ (n = 2). Finally, one-way ANOVAs~~)~~ were also run for each pCO$_2$ level to investigate differences in sediment responses across temperatures (n = 4). Post-hoc Tukey's tests were then used to determine which temperatures had similar or different responses. ~~Finally, a two-way ANOVA was conducted on each dataset to identify whether there were interacting effects on O$_2$, DIC and DOC fluxes of the combined stressor condition, temperatures (n = 4) and/or CO$_2$ concentrations (n = 2).~~

2.5.2 Diel variability with temperature for DIC and DOC fluxes

Differences between dark and light cycles were compared to further investigate changes observed in DIC and DOC net variability. Similar analyses to those described above were applied~~ here~~. To examine differences among temperatures (n = 4), light-condition (n = 2~~)~~), and whether light-condition significantly interacted with temperature response, two-way ANOVAs were applied to current and ~~future~~high-$p\text{CO}_2$ cores, separately. Following this, each light-condition was further investigated to consider the individual temperature responses in the light and dark separately using one-way ANOVAs and Post-hoc Tukey's tests.

3 Results

3.1 Overlapping control scaling

Mean rates calculated from overlapping control cores present in each week were compared to establish whether the two sets of incubations were directly comparable, and whether changes attributed to ~~future~~high-$p\text{CO}_2$ were truly due to that treatment, and not just a temporal shift in how the sediments were behaving. The P/R ratios were similar for incubations (0.84 ± 0.01 and 0.83 ± 0.04, respectively), however, the magnitude of the R and NPP fluxes ~~were~~was ~23 % greater for control cores in the ~~future~~high-$p\text{CO}_2$ week (Table S5; discussed in Sect. 4.0). As such, R and NPP rates of cores were scaled to mean control rates (n = 6) using the proportional rate difference calculated between the treatments and the individual controls present in the respective weeks (n = 3) (Eq. (~~7~~6)). Scaled rates were within ± 13 % of actual rates. There were no significant differences between controls for light or dark production of DIC or DOC.

3.2 Productivity and respiration responses to OA

~~Future~~High-$p\text{CO}_2$ alone (~~under~~at mean ambient ~~temperature~~temperatures, 23 °C) significantly increased P/R by ~20 % over control ratios (~~paired-sample: t = -14.14~~one-way: $F_{3,4} = 101.9$, p = 0.~~005, df = 2~~0005; Figure 2d). This was a result of significant increases in NPP (~42 %) ~~from~~compared to control conditions (~~paired-sample: t = -7.57~~one-way: $F_{3,4} = 241.4$, p ~~=~~ <0.~~017, df = 2~~0005; Figure 2b), in concert with no significant change in R (~~paired-sample: t = 2.68~~one-way: $F_{3,4} = 4.5$, p = 0.~~12 df = 2~~10; Figure 2a). ~~Similarly, significant~~Insignificant increases of DIC uptake in the light reflected the significant increases in NPP with ~~future~~high-$p\text{CO}_2$ at ambient ~~temperatures (paired-sample: t = -18.88~~temperature (one-way: $F_{3,4} = 5.9$, p = 0.~~003, df = 2~~07; Figure 3c). Like R, DIC in the dark did not change with $p\text{CO}_2$ (~~paired-sample: t = 0.32~~one-way: $F_{3,4} = 1.3$, p = 0.~~78, df = 2~~33; Figure 3b). GPP also significantly increased with ~~OA~~high-$p\text{CO}_2$ at ambient temperatures (~~paired-sample: t = -5.70~~one-way: $F_{3,4} = 65.3$, p = 0.~~03, df = 2~~001; Figure 2c), with net DIC significantly shifting from a slight efflux to a slight influx (~~paired-sample: t = -6.91~~one-way: $F_{3,4} = 24.3$, p = 0.~~02, df = 2~~008; Figure 3a).

3.3 Productivity and respiration responses to temperature and OA

~~R~~Temperature had a strong effect on R, NPP, GPP and P/R~~ had strong responses to temperature with OA~~, whereas only ~~affecting~~ light cycle NPP and in turn, GPP and P/R~~. R~~ were affected by OA.

The response of R to temperature was similar at both current and ~~future~~high-$p\mathrm{CO}_2$ (no two-way interaction: $F_{3,16} = 0.77$, $p = 0.53$; Figure 2a), ~~with no effect of~~ and was not affected by $p\mathrm{CO}_2$ ~~on R response~~ (CO_2 effect two-way: $F_{1,16} = 0.99$, $p = 0.34$; Figure 2a). Accordingly, Q_{10} values ~~between $p\mathrm{CO}_2$ conditions~~ for R were similar~~, 1.66 and 1.69~~ for current (1.66) and ~~future~~high-$p\mathrm{CO}_2$~~, respectively~~ (1.69) (Table 2). ~~R increased~~R changed significantly across the 8 °C temperature range, increasing by ~11 % and ~29 % in higher temperature cores (Δ+3 °C and Δ+5 °C, respectively) and ~~decreased~~decreasing by ~16 % in Δ-3 °C cores (temperature effect two-way: $F_{3,16} = 36.93$, $p < 0.0001$; Figure 2a).

Sediment NPP~~ response of sediments~~ was significantly affected by the interaction of $p\mathrm{CO}_2$ and temperature (two-way interaction: $F_{3,16} = 8.92$, $p = 0.001$; Figure 2b). Under current-$p\mathrm{CO}_2$, NPP ~~response was~~decreased significantly~~ decreased~~ with increased temperature (one-way: $F_{3,8} = 41.94$, $p < 0.0001$; Figure 2b), ~~with NPP rates~~ shifting from net ~~autotrophic~~autotrophy in the light in low and control temperature cores (efflux of 590 ± 121 and 613 ± 10 μmol-O_2 m^{-2} h^{-1}, respectively) to net ~~heterotrophic~~heterotrophy in higher temperature cores (influx of 163 ± 228 and 390 ± 97 μmol-O_2 m^{-2} h^{-1}, for Δ+3°C and Δ+5°C respectively). Warming alone therefore resulted in a reduction in ~~rates~~NPP by 126 % at Δ+3 °C and 164 % at Δ+5 °C, compared to the control (Figure 2a). ~~In contrast, NPP response to temperature under future-$p\mathrm{CO}_2$ maintained net autotrophy in the light at Δ+3 °C and only resulted in net heterotrophy in the highest temperature treatments (one-way: $F_{3,8} = 53.01$, $p < 0.0001$; Figure 2b). Although~~ OA in general significantly increased NPP rates over those measured under current-$p\mathrm{CO}_2$ conditions (CO_2 effect, two-way: $F_{1,16} = 21.92$, $p = 0.0003$; Figure 2b), and Q_{10} of NPP increased from 1.13 to 1.92 ~~(Table 2), the NPP response to~~when OA ~~at Δ+3 °C, reflecting stimulation of primary production, allowed sediments to remain~~was present (Table 2). As such, under high-$p\mathrm{CO}_2$ NPP maintained net ~~autotrophic~~autotrophy in the light ~~instead of shifting to~~at Δ+3 °C and only resulted in net heterotrophy ~~as they did under current-$p\mathrm{CO}_2$ (Figure 2~~in the highest temperature treatment (one-way: $F_{3,8} = 53.01$, $p < 0.0001$; Figure 2b).

GPP ~~reflected~~displayed a similar interactive stressor response to that described for NPP (two-way interaction: $F_{3,16} = 9.39$, $p = 0.0008$; Figure 2c). Under current-$p\mathrm{CO}_2$, GPP had a slight, but insignificant rate increase from lowered to control temperatures (~12 %), where rates significantly decreased at temperatures higher than control (~45 % and ~50 % for Δ+3 °C and Δ+5 °C, respectively) (one-way: $F_{3,8} = 16.89$, $p = 0.001$; Figure 2c). OA significantly increased GPP~~ rates~~ at ambient and Δ+3 °C temperatures (CO_2 effect, two-way: $F_{1,16} = 24.77$, $p = 0.0001$; Figure 2c), resulting in a stronger temperature ~~response of future~~sensitivity in GPP under high-$p\mathrm{CO}_2$ ~~sediments~~conditions (one-way: $F_{3,8} = 40.90$, $p < 0.0001$; Figure 2c) than under current-$p\mathrm{CO}_2$ ~~sediments (one-way: $F_{3,8} = 16.89$,~~ ($p = 0.001$~~; Figure 2c).~~). This increased sensitivity of GPP to temperature ~~dependence increase~~ was supported by GPP Q_{10} value differences between current and ~~future~~high-$p\mathrm{CO}_2$ conditions, increasing from 1.46 to 2.27 (Table 2).

The differences in P/R among treatments further highlighted significant interaction of temperature and $p\mathrm{CO}_2$ (two-way interaction: $F_{3,16} = 5.86$, $p = 0.007$; Figure 2d), suggesting ~~GPP responses to~~ the effect of $p\mathrm{CO}_2$ ~~were~~on primary productivity was strong enough to alter the overall productivity of the sediments. Under current-$p\mathrm{CO}_2$, ~~GPP rates had a slight, but~~

~~insignificant rate increase from lowered to control temperatures (~12 %), where rates significantly decreased at temperatures higher than control (~45 % and ~50 % for ∆+3 °C and ∆+5 °C, respectively; Figure 2c). As such,~~ P/R reflected GPP with a clear separation between control and ∆-3 °C sediments having a higher P/R (0.84 ± 0.01 and 0.89 ± 0.07, respectively) than the significantly lower ratios ~~(one-way: $F_{3,8}$ = 49.41, p <0.0001; Figure 2d)~~ calculated in increased temperature cores (0.42 ± 0.11 and 0.33 ± 0.05 for ∆+3 °C and ∆+5 °C, respectively~~).~~) (one-way: $F_{3,8}$ = 49.41, p <0.0001; Figure 2d). Similarly, under ~~future~~high-$p\mathrm{CO}_2$, the effect of GPP on P/R was clear. The positive effect of ~~OA~~high-$p\mathrm{CO}_2$ conditions on GPP ~~response~~ pushed the P/R ratio of ∆-3 °C and control temperature cores to ~1 (1.09 ± 0.16 and 1.03 ± 0.03, respectively), suggesting the ecosystem shifted ~~to~~toward net autotrophy under those conditions. The positive effect of ~~OA~~high-$p\mathrm{CO}_2$ was also highlighted at ∆+3 °C, with P/R (0.77 ± 0.13) remaining close to current ecosystem ratio (0.84 ± 0.01) instead of significantly dropping like those calculated under current-$p\mathrm{CO}_2$ or in ∆+5 °C cores (0.25 ± 0.04, one-way: $F_{3,8}$ = 38.58, p <0.0001; Figure 2d).

3.4 DIC fluxes

DIC fluxes mirrored those of dissolved oxygen (Figure 3 and Figure 2) with both light and dark DIC:DO ratios near 1:1 (Figure 4). In the dark, DIC reflected R responses to temperature; like R, DIC responses to temperature did not differ with $p\mathrm{CO}_2$ (two-way interaction: $F_{3,16}$ = 0.92, p = 0.45; Figure 3b) and rates increased with increasing temperature (temperature effect two-way: $F_{3,16}$ = 12.66, p = 0.0002; Figure 3b). In the light, there was a significant interactive effect of temperature and $p\mathrm{CO}_2$ on DIC fluxes (two-way interaction: $F_{3,16}$ = 12.01, p = 0.0002; Figure 3c). Under current-$p\mathrm{CO}_2$, DIC reflected the significant NPP responses to temperature, with DIC taken up at ∆-3 °C and control temperatures and effluxed at ∆+3 °C and ∆+5 °C (one-way: $F_{3,8}$ = 21.33 p = 0.0004; Figure 3c).

Net DIC responses were significantly affected by the interaction of $p\mathrm{CO}_2$ and temperature (two-way interaction: $F_{3,16}$ = 9.69, p = 0.001; Figure 3a). Like differences in O_2, significant differences between $p\mathrm{CO}_2$ conditions were also measured in the ∆+3 °C temperature cores. At ∆+3 °C, net DIC production in ~~future~~high-$p\mathrm{CO}_2$ cores was ~62 % lower than that measured at the same temperature under current-$p\mathrm{CO}_2$ (~~paired-sample: t = 5.82, df = 2~~one-way: $F_{3,4}$ = 17.1, p = 0.~~03~~01; Figure 3a). This again reflected changes in light cycle production, with light DIC effluxes at ∆+3 °C under current-$p\mathrm{CO}_2$ becoming influxes under ~~future~~high-$p\mathrm{CO}_2$ (132 ± 74 μmol-C m^{-2} h^{-1} to -617 ± 88 μmol-C m^{-2} h^{-1}, respectively; Figure 3a).

3.5 DOC fluxes

At current-$p\mathrm{CO}_2$, increasing temperature resulted in a significant shift in net DOC fluxes, going from effluxes at the two lower temperatures (∆-3 °C and control) to uptakes at the two higher temperatures ~~at current-$p\mathrm{CO}_2$~~ (one-way: $F_{3,8}$ = 6.96, p = 0.013; Figure 5a). The relative light and dark cycle contributions of these net trends at current-$p\mathrm{CO}_2$ were also affected by temperature (two-way interaction: $F_{3,16}$ = 13.18, p = 0.0001; Figure 5b). Significant changes in DOC fluxes in the dark shifted from an efflux at ∆-3 °C to an uptake at control temperature, with higher uptake rates at ∆+5 °C (26 % higher than control rates; one-way dark: $F_{3,8}$ = 8.64, p = 0.007; Figure 5b). In contrast, the highest DOC effluxes in the light were at control temperatures, significantly decreasing with both increasing and decreasing temperatures to DOC fluxes around zero (one-way: $F_{3,8} = 16.76$, p = 0.001; Figure 5b).

~~OA~~High-$p\mathrm{CO_2}$ alone (at ambient mean temperatures, 23 °C) had a significant effect on net DOC, shifting from a slight efflux at current-$p\mathrm{CO_2}$ (~0.5 mmol-C m^{-2} d^{-1}) to a significant uptake at ~~future~~high-$p\mathrm{CO_2}$ (~10.9 mmol-C m^{-2} d^{-1}; ~~paired-sample: t = 5.74, df = 2~~one-way: $F_{3,4} = 25.1$, p = 0.~~03~~007; Figure 5a). The trend in temperature response was similar for ~~future and~~ current and high-$p\mathrm{CO_2}$ (two-way interaction: $F_{3,16} = 0.88$, p = 0.47; Figure 5a), but there was a significant shift from small efflux at lower temperatures to considerable uptakes at all temperatures with high-$p\mathrm{CO_2}$ (two-way $\mathrm{CO_2}$ effect: $F_{1,16} = 61.46$, p <0.0001; Figure 5a). Differences between dark and light DOC fluxes under high-$p\mathrm{CO_2}$ were~~ also~~ independent of temperature (two-way interaction: $F_{3,16} = 1.94$, p = 0.16; Figure 5c~~)~~), with the overall magnitude of ~~fluxes~~influxes in the dark being significantly greater than those in the light (two-way light-condition: $F_{1,16} = 15.83$, p = 0.001; Figure 5c). Loss of statistically different temperature responses for high-$p\mathrm{CO_2}$ light and dark responses (temperature effect two-way: $F_{3,16} = 1.05$, p = 0.40; Figure 5c) was in large part due to within treatment variability in the ~~future~~high-$p\mathrm{CO_2}$ cores.

4 Discussion

~~The aim of this study was to explore the changes in DOC demand and production in unvegetated estuarine sediments under a range of temperatures at current and future $p\mathrm{CO_2}$ levels. The purpose of this was to gain a better understanding of how unvegetated sediments contribute to estuarine DOC export and how this will change under projected future climate conditions. An important component of the~~An important component of this study was testing the interaction and individual effects of warming and OA on DOC processing. This was necessarily achieved through a comparison of core incubations occurring in different weeks. As such, it is important to consider the limitations of this approach. Control treatments in different weeks would ideally be the same in all respects, but there were some differences. For instance, NPP and R were higher in the incubation week for current-$p\mathrm{CO_2}$ conditions (Table S5), likely due to small changes in environmental conditions, e.g. salinity differences (24 versus 17.7 for current and ~~future~~high-$p\mathrm{CO_2}$, respectively; Table 1). ~~Yet~~However, these differences did not significantly affect DOC fluxes, nor the heterotrophy of the sediments (P/R = 0.84 ± 0.01 and 0.83 ± 0.04; Table S5). Moreover, sediments in separate weeks maintained the same OM content (~3.5 %) and molar C:N ratio (~16), suggesting that differences in processing have very little short-term impact on the overall OM pool in the sediment due to the OM pool size being about 3 orders of magnitude higher than any diel flux (organic carbon pool ~12,000 mM). Thus, because all conditions in the laboratory setup were the same for each incubation (with the exception of $p\mathrm{CO_2}$ in treatment tanks, which was intentionally manipulated to be different) the difference in fluxes between controls were attributed to differences in when the sediments and overlying waters were collected. Therefore, the scaling of NPP and R (Table S6) were done for the sake of treatment comparison, resulting in scaled rates within 13% of actual measured values, which had a negligible effect on P/R (< 1 % across all treatments). The final NPP and R rates in comparisons across treatments should thus be considered relative to control rates and be interpreted as approximate values (± 13 %).

Understanding current ecosystem functioning is of primary interest when trying to determine how disturbances in the environment may change metabolic rates and pathways of OM mineralization (Jørgensen, 1996; D'Avanzo et al., 1996; Malone and Conley, 1996). ~~The~~Based on unadjusted R rates, the near 1:1 ratio of DIC production to O_2 consumption in the dark (respiratory quotient, RQ of ~1.13 ± 0.05; Figure 4) suggests that aerobic respiration dominated the sediments (Eyre and Ferguson, 2002). Similarly, unadjusted NPP rates suggest that aerobic processes dominated ~~the~~ benthic production in the light ~~as shown by the~~, with a 1:1 ratio of O_2 and DIC fluxes (Fig. 4; Eyre and Ferguson, 2002). Sediments ~~here~~in the current study were net heterotrophic with a P/R in control cores of ~0.84 ± 0.01 and ~0.83 ± 0.04 during current and ~~future~~high-$p\mathrm{CO}_2$ incubation weeks, respectively. Despite the undeniable range of P/R ratios unvegetated estuarine sediments may experience (1.2 to 0.01 in Oakes et al., 2012; and Ferguson and Eyre, 2013, respectively), the ratios ~~here~~in the current study were similar to mean global ~~model~~ estimates for unvegetated estuarine sediments (~0.82, calculated from values in Duarte et al., 2005) and calculated from P ~~an~~and R values of 22 estuaries globally ~~(~0.84, compiled by Smith and Hollibaugh, 1993), suggesting that the metabolic function of sediments in the current study are representative of estuarine sediments globally and the impacts observed in this study should be broadly applicable.~~(~0.87, compiled by Smith and Hollibaugh, 1993), suggesting that the metabolic function of sediments in the current study are representative of estuarine sediments globally and the impacts observed in this study should be broadly applicable.

4.1 DOC fuels benthic respiration

DOC appeared to be a significant driver of benthic respiration (Figure 5b). At control temperatures (23 °C) net DOC fluxes were near zero (0.47 ± 0.93 mmol-C m^{-2} d^{-1}), indicating that the diel production and uptake of DOC across the sediment-water interface was balanced (Figure 5a). The control rates in the present study were close to benthic DOC flux rates reported for subtropical estuarine sediments in most seasons, ~1.5 mmol-C m^{-2} d^{-1}, except summer (Maher and Eyre, 2010). Relative to our control (summer) rates, Maher and Eyre (2010) reported higher net DOC flux rates (~10 mmol-C m^{-2} d^{-1}) as a result of DOC effluxes in both the light and dark (Maher and Eyre, 2010). ~~Our~~We observed similar light DOC ~~fluxes~~effluxes (610 μmol-C m^{-2} h^{-1})~~ were similar~~ to those of Maher and Eyre (2010) in summer (~647 μmol-C m^{-2} h^{-1}~~). The difference~~), whereas uptake of DOC in the ~~DOC processing~~ dark in the ~~sediments came from dark uptake~~ current study (-571 μmol-C m^{-2} h^{-1}~~) versus~~), Maher and Eyre (2010) reported dark ~~efflux in the previous study~~DOC effluxes (254 μmol-C m^{-2} h^{-1}, Maher and Eyre, 2010). This release of DOC in the dark was attributed to enhanced microbial coupling in the sediments under warmer temperatures (Maher and Eyre, 2010)~~, yet here~~. In the current study, and in previous reports, DOC uptake suggests that bacteria not only intercepted DOC produced from within the pore waters (potentially satisfying up to 60 % of total mean bacterial production, Boto et al., 1989), but also took up available DOC from the water column to satisfy its metabolic requirements (Boto et al., 1989; Brailsford et al., 2019), effectively acting as a DOC sink. Under conditions of reduced light availability and/or intensity, sediments are expected to have an amplified heterotrophic response in addition to a reduction in microalgal production of DOC.

4.2 OA increases DOC ~~uptake~~ assimilation

Positive responses in primary production were associated with OA. The ~72 % increase in NPP rates at ambient temperatures ~~were~~was consistent with general stimulation of primary production in finer sediments with increased DIC availability (Vopel et al., 2018; Oakes and Eyre, 2014). Sediments may become DIC-limited when algal demand is relatively high compared to porewater supply of CO_2 (Cook and Røy, 2006), and MPB therefore may benefit from an increase in CO_2 availability. MPB in fine sediments are restricted to dissolved substrates (i.e., nutrients and DIC) accessed via diffusion from deeper and adjacent sediments, and the overlying water column (Boudreau and Jørgensen, 2001). This makes them more likely to deplete accessible DIC than MPB in permeable sediments. Primary producers in permeable sediments, like those in reef ecosystems, therefore do not often experience the same increase in primary production with increased CO_2 (Trnovsky et al., 2016; Cyronak and Eyre, 2016; Eyre et al., 2018; Cook and Røy, 2006; Vopel et al., 2018). As well as differences in diffusive versus advective modes of solute transfer between the sediment types (Cook and Røy, 2006), ~~but~~ variable response may also ~~may~~ be ~~partially due~~attributable to sandier sediments being limited by other factors such as nutrient and OM availability~~ as they~~, given that coarser sediments are generally more oligotrophic (Admiraal, 1984; Heip et al., 1995). ~~Therefore, DIC limitations to~~ In comparison, nutrients were non-limiting in the less permeable sediments used in the current study, based on nutrient concentrations that increased during all incubations (see supplementary methods and Table S7). MPB growth rates ~~are likely higher under~~in sediments with low ~~sediment~~ permeability ~~like those here and primary~~are more likely limited by DIC availability. Primary productivity responses to pCO_2 would likely differ in permeable sediments where general access to CO_2 is greater.

Given that MPB exude carbon (Maher and Eyre, 2010), we would expect increased GPP to correspond with increased DOC production and flux. However, although OA stimulated primary production (Figure 2), we instead saw increased DOC uptake in the dark (Figure 5). A likely explanation is that bacterial uptake of DOC was stimulated through the provision of labile carbon from MPB (Morán et al., 2011; Hardison et al., 2013). As such, DOC appeared to fuel much of the dark cycle respiration, as DOC uptake in the dark reflected dark DIC production (respiration), except for sediments at Δ-3 °C under current-pCO_2. ~~Under current-pCO_2, the sediment uptake of DOC in the dark accounted for ~50 % of the total respired DIC. This suggests that there was respiration of other carbon sources, potentially more refractory DOC sourced from within the pore waters (Boto et al., 1989), with possibly more metabolic energy invested for the production of ectoenzymes needed to hydrolyze this more refractory DOC (Chróst, 1990; Chróst, 2017). The portion of DIC accounted for by dark DOC increased from 50 to 100 % under the future-pCO_2 climate. In part, this may be due to the increase in available labile organic carbon (Moran and Hodson, 1990) arising from the increase in NPP under future-pCO_2 across all temperatures (Figure 2b). The increase in the ratio of DOC uptake to DIC efflux from 0.5 to 1.0 may be due to the bacteria no longer needing to synthesise ectoenzymes in the presence of readily utilizable organic carbon (Chróst, 1992; Chróst, 2017), resulting in a~~Under current-pCO_2, uptake of DOC in the dark accounted for only ~50 % of the DIC respired in the dark. The portion of DIC accounted for by dark DOC uptake increased from 50 to 100 % under the high-pCO_2 conditions. In part, this may have been due increased availability of labile organic carbon (Moran and Hodson, 1990) arising from the increase in NPP under high-pCO_2 across all temperatures (Figure 2b), which would reduce the need for bacteria to synthesise ectoenzymes (Chróst, 1992; Chróst, 1991), resulting in more rapid turnover of carbon to the water column.

4.3 Warming drives increased heterotrophy and DOC assimilation ~~increases~~

Sediments in this study, like other manipulative studies~~,~~ in both permeable sands (Lantz et al., 2017; Trnovsky et al., 2016) and cohesive sediments (Apple et al., 2006), demonstrated increased heterotrophy with increased temperature. This shift to heterotrophy is often attributed to the imbalance in the thermal sensitivity of heterotrophic over autotrophic ~~metabolic thermal sensitivity~~ metabolism (Yang et al., 2016; Allen et al., 2005). More specifically, differences in activation energy dictated by differences in physiology and biochemical processes (Patching and Rose, 1970; Apple et al., 2006) result in ~~greater~~ increases in heterotrophic activity with increasing temperature than ~~autotrophic~~exceed increases in autotrophic activity (Yang et al., 2016). However, in this study, under current-$p\mathrm{CO}_2$, the increases in R and GPP from Δ-3 °C to control temperatures were similar (~16 % and ~11 %, respectively), whereas at higher temperatures, GPP decreases far exceeded increases in R (7~~ times~~ × and 3~~ times,~~×, for 26 °C and 28 °C respectively). Therefore, unlike previous studies, decreases in MPB productivity at higher temperatures appeared to be a greater driver towards heterotrophy than increases in respiration rates. In other words, temperature increases not only increased the rate of DOC uptake, but also likely decreased the rate of DOC production.

4.3.1 Warming reduces GPP and DOC production under current-$p\mathrm{CO}_2$

Primary production is the main source of DOC in marine ecosystems (Wagner et al., 2020). Decreasing trends in GPP with warming under current-$p\mathrm{CO}_2$ seen here have been described previously where photosynthetic growth and production decline at higher temperatures (Thomas et al., 2012). Photosynthetic productivity is often linked to seasonal temperature (Apple et al., 2006), which is also associated with differences in environmental factors such as light, nutrient concentrations, and DOM quality and availability (Geider, 1987; Herrig and Falkowski, 1989). Although the relative availability of light and nutrients do influence productivity rates (Kana et al., 1997) and would be expected to influence in situ seasonal production, the current study controlled light and initial nutrient concentrations in the water column to isolate the effect of temperature. Thus, decreasing GPP was driven by warming, suggesting that MPB in these subtropical sediments likely had a temperature optimum around current mean summer temperatures of ~23 °C (GPP: 1515 ± 37 μmol-O_2 m^{-2} h^{-1}; Figure 2~~c).~~c). Longer-term warming could allow for possible migration of more tolerant species to settle from lower latitudes (Hallett et al., 2018), shifting the composition of the benthic community. The introduction of more tolerant species could reduce the increase in heterotrophy and net DOC removal from the water column seen here. However, the species diversity of the estuarine sediments will ultimately decrease as they are pushed to temperature extremes (Thomas et al., 2012), reducing the functional redundancy of the microbial community. This decreased functional redundancy has the potential to make unvegetated estuarine sediments less resilient to environmental perturbations under future climate conditions.

4.3.2 Warming increases respiration and DOC ~~uptake~~assimilation

Unlike photosynthetic productivity, heterotrophic respiration often has a linear rate increase with temperature to the thermal optimum due to heterotrophs not being constrained by the same abiotic variables (e.g., nutrient and light availability) as primary

~~production~~producers (Apple et al., 2008; Apple et al., 2006; Geider, 1987; Yap et al., 1994). In this study, respiration rates under both current and ~~future~~high-$p\mathrm{CO}_2$, increased from lowest rates measured at Δ-3 °C to maximum rates (>50 % greater) at Δ+5 °C (Figure 2a). Consistent with overall lower respiration rates relative to other subtropical unvegetated sediments (~900 to ~1500 μmol-O$_2$ m^{-2} h^{-1}, Ferguson and Eyre, 2013) the temperature dependence of respiration under both current and ~~future~~high-$p\mathrm{CO}_2$ conditions (Q_{10} = 1.66 and 1.69, respectively) was slightly lower than is typical for biological systems (Q_{10} = 2, Valiela, 1995), but similar to temperature dependence described in other estuarine systems (Q_{10} = 1.5-1.9, Morán et al., 2011), with values towards the lower end of this range possibly being a result of resource limitation (López-Urrutia and Morán, 2007).

A potential limiting resource for bacteria in estuarine sediments is dissolved organic matter (DOM) (Church, 2008), ultimately controlling the flow of carbon through the microbial loop (Kirchman and Rich, 1997). However, in the presence of sufficient DOM, warming has been associated with increased bacterial DOM incorporation (Kirchman and Rich, 1997). In line with this, ~~an~~ increased uptake of DOC at higher temperatures and efflux at lower temperatures was observed. Although DOC is mainly produced by photoautotrophs, DOC can be produced in the dark ~~(i.e., cell lysis via viruses and potential bacterial grazing via meograzer, Carlson, 2002).~~through, for example, chemodegradation of detrital organic carbon and cell lysis by viruses and during grazing (Carlson, 2002). As such, the efflux of DOC in the dark at Δ-3 °C suggests that heterotrophic bacterial productivity, and therefore DOC uptake, was reduced by lowered temperatures (Raymond and Bauer, 2000), resulting in a failure to intercept all DOC produced in the pore waters. This failure to intercept DOC may be compounded if nutrient supply is limited (Brailsford et al., 2019), as it is common for heterotrophic bacteria to rely on refractory DOC ~~under such conditions (Chróst, 2017).~~when labile sources are not readily available (Chróst, 1991), which can occur under conditions of nutrient limited biological productivity (Allen, 1978)..

4.3.3 Global estuarine loss of DOC from unvegetated sediments in the future

~~Up to 3.5 times more DOC reaches the ocean interior from coastal areas than the open ocean (Costanza et al., 1997).~~Up to 3.5× more DOC reaches the ocean interior from coastal areas than the open ocean (Duarte, 2017; Krause-Jensen and Duarte, 2016; Hansell et al., 2009). As such, small changes to the coastal export of DOC may have a disproportionately large influence on the global DOC budget. Our findings suggest a reduced export of DOC to the ocean from the coastal zone under ~~future~~high-$p\mathrm{CO}_2$ conditions, across the full 8 °C temperature range ~~in~~due to changes in carbon processing within unvegetated sediments. Despite the lack of seasonality in the study, the inclusion of an 8 °C temperature range, including temperatures below current mean temperatures, suggests that seasonal temperature variation is unlikely to have a significant effect on the relative change in DOC in the future (Figure 5). Although any upscaling of a single controlled experiment to a global scale is highly speculative, we ~~feel it is better to include an estimate to demonstrate the potential changes that may transpire under a future high-$p\mathrm{CO}_2$ climate and the potential importance of unvegetated sediments in DOC export from coastal zones by putting our findings in a global context, than not to attempt an estimate at all. The following estimates should be considered in this context. Moreover, we~~believe it is valuable to demonstrate the potential for a high-$p\mathrm{CO}_2$ climate to cause globally significant change in DOC export from coastal zones. Furthermore, putting our findings in a global context, provides a guideline value for potential change. The following estimates should be considered in this context and it should be expected that different hydrodynamic settings, sediment and/or sediment community composition, and sources of organic matter could affect the outcome. For example, the response to warming and pCO_2 may be different for pelagic communities and/or in deeper waters that are subject to stratification (Li et al., 2020), where access to nutrients and CO_2 may become limiting (Rost et al., 2008). We have applied our results to global coastal DOC exports (Maher and Eyre, 2010; Duarte, 2017) as an initial step in ~~modelling~~estimating responses of unvegetated sediment habitats to future high-pCO_2 climate. We do not assume that the responses of unvegetated sediments to the future climate found here are applicable to other ecosystems dominated by macrophytes, and thus did not apply our findings to vegetated coastal habitats.

To estimate total DOC export from coastal zone under a future high-pCO_2 climate of Δ+3 °C and OA, the sediment uptake rate of 19 ± 4 mmol-C m^{-2} d^{-1} was scaled to the global surface area of unvegetated estuarine sediments (1.8×10^{12} m^2; Costanza et al., 1997). ~~An~~On this basis, an estimated 150 Tg-C would be removed from the coastal zone by unvegetated estuarine sediments annually~~.~~ under OA conditions with an accompanying 3°C temperature increase. To then calculate the potential impact of this uptake, we applied our estimates to existing future global coastal DOC export estimates (Maher and Eyre, 2010; Duarte, 2017). Mean benthic DOC export from estuaries, including intertidal and vegetated habitats, has been estimated at 168 Tg-C yr^{-1} (90-247 Tg-C yr^{-1}) (Maher and Eyre, 2010). Under this scenario, the switch to DOC uptake by sediments under future climate conditions (Figure 5a) would result in ~~an~~ ~90 % reduction in total mean ~~benthic~~ estuarine DOC export (Maher and Eyre, 2010), decreasing the load from ~168 Tg-C yr^{-1} to ~18 Tg-C yr^{-1}. Other global estimates of DOC exported from coastal vegetated ecosystems range from 114 up to 1,853 Tg-C yr^{-1} (Duarte, 2017), with ~~model~~scaled estimates suggesting unvegetated estuarine sediments may consume 8 to 132 % of this DOC under a future high-pCO_2 climate. As such, this basic ~~modelling~~upscaling suggests that by impacting DOC fluxes in unvegetated sediments, future climate conditions ~~could~~have the potential to significantly impact global DOC export from coastal systems to the open ocean. This has implications for global marine productivity and carbon transfer to the ocean interior (Krause-Jensen and Duarte, 2016). However, to get a more accurate insight into global carbon cycling the response of DOC export from estuarine vegetated habitats to future climate also needs to be studied.

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Data availability

Archived data will be available for access on PANGAEA upon publication (currently under review). Until then, data available upon request.

Author contributions

All listed authors have contributed substantially to preparation and drafting of this paper and have approved the final submitted manuscript. Specifically, MS conceived the project, collected data, ran data analysis and interpretation, and led the writing of the manuscript. KS, JO, and BE helped conceive the project, contributed to interpretation and helped draft the manuscript.

Competing interests

The authors declare that they have no conflict of interest

Acknowledgements

Thanks are extended to P. Kelly, I. Alexander, M. Carvalho, N. Carlson-Perret, J. Yeo, and N. Camillini for their assistance in the field and support in the laboratory. Special thanks to Z. Kennedy allowing access to their property for sample collection. This work was supported in the form of an SESE Postgraduate Scholarship from Southern Cross University, Lismore, NSW, Australia and ARC Discovery projects DP150102092 and DP160100248.

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Table 1. Start conditions for current and ~~future~~ high-$p\text{CO}_2$ (*) incubations showing mean (±SD) of various carbonate parameters. CON* is the overlapping control core present in the ~~future~~high-$p\text{CO}_2$ incubation week.

Scenario	**Sal** (‰)	**T** (°C)	**pH** (Free Scale)	$\boldsymbol{p}\textbf{CO}_2$ (μatm)	$\textbf{HCO}_3^-$ (μmol/ kgSW)	$\textbf{CO}_3^{2-}$ (μmol/ kgSW)	**TA** (μmol/ kgSW)	**DIC** (μmol/ kgSW)
Δ-3 °C	24.4	21.0 (±0.1)	8.08 (±0.02)	453.1 (±24.0)	1750.8 (±2.9)	123.9 (±6.4)	2048.7 (±12.6)	1889.8 (±3.3)
*	17.7	20.8 (±0.1)	7.60 (±0.02)	989.8 (±40.7)	1232.9 (±2.6)	24.28 (±0.9)	1293.8 (±2.8)	1291.6 (±2.9)
Control	24.4	23.1 (±0.0)	8.07 (±0.02)	469.9 (±27.2)	1744.7 (±4.1)	130.2 (±6.9)	2056.9 (±12.1)	1889.6 (±2.0)
*	17.7	23.2 (±0.1)	7.63 (±0.06)	995.9 (±146.6)	1281.6 (±5.5)	29.5 (±4.3)	1354.7 (±12.7)	1343.4 (±6.1)
Δ+3 °C	24.4	25.6 (±0.5)	8.08 (±0.01)	471.5 (±13.3)	1723.5 (±2.0)	136.9 (±3.4)	2051.6 (±6.2)	1874.5 (±1.5)
*	17.7	25.8 (±0.2)	7.64 (±0.12)	1011.1 (±248.6)	1265.8 (±2.7)	32.7 (±9.3)	1346.7 (±23.6)	1329.2 (±3.4)
Δ+5 °C	24.4	27.1 (±0.1)	8.11 (±0.05)	445.2 (±56.2)	1698.3 (±22.7)	155.3 (±17.0)	2069.3 (±17.6)	1866.1 (±7.4)
*	17.7	27.9 (±0.1)	7.65 (±0.12)	989.6 (±40.7)	1254.4 (±5.2)	34.3 (±1.3)	1339.2 (±3.8)	1317.1 (±5.2)
CON*	**17.7**	**23.3** **(±0.1)**	**7.96** **(±0.05)**	**431.9** **(±45.7)**	**1193.0** **(±4.1)**	**58.4** **(±6.1)**	**1338.2** **(±10.9)**	**1265.5** **(±1.1)**

Table 2. $\textbf{Q}_{10}$ and $\textbf{T}_{opt}$ values for current and ~~future~~high-$p\text{CO}_2$ climates

	R		**NPP**		**GPP**	
	Current	**~~Future~~High**	**Current**	**~~Future~~High**	**Current**	**~~Future~~High**
Q_{10}	1.66	1.69	1.13	1.92	1.46	2.27
T_{opt} (°C)	28	28	23	23	23	23

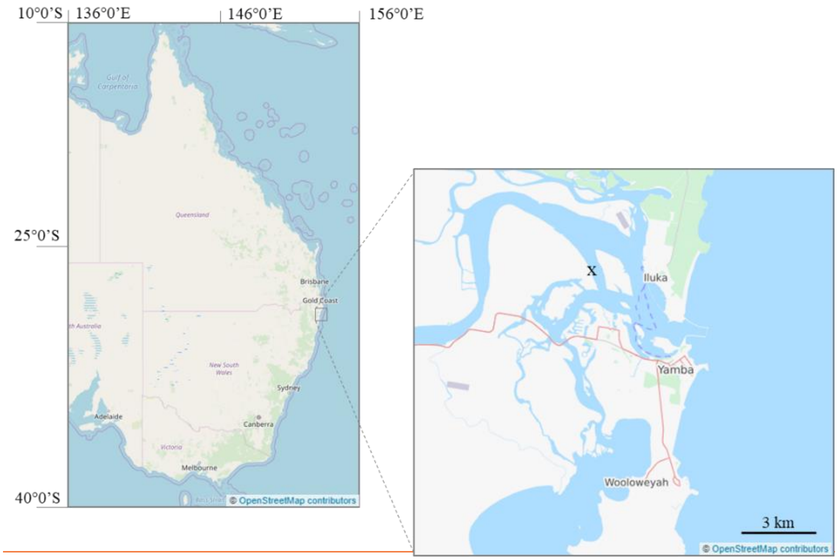
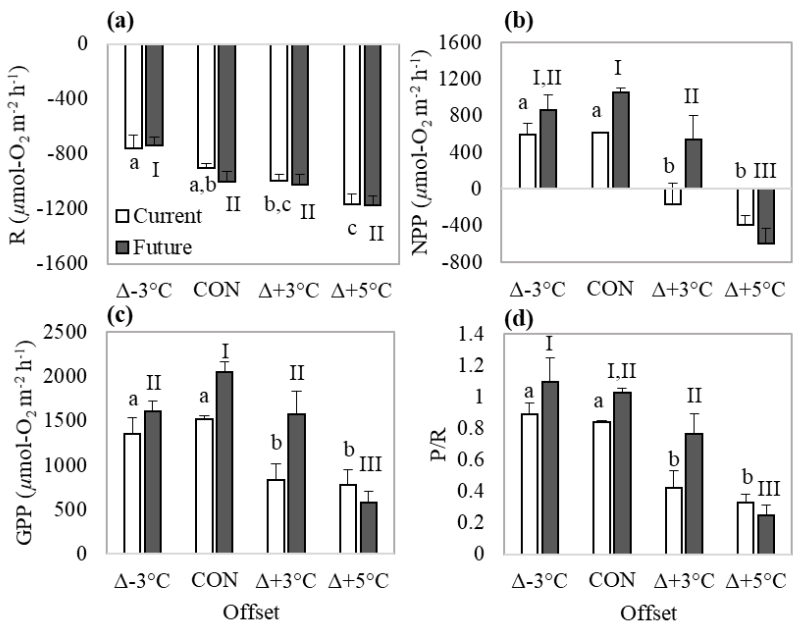


Figure 1. Study location (x; 29°24.21'S, 153°19.44'E) marked on a map of Yamba, NSW embedded in an east coast map of Australia. © OpenStreetMap contributors 2020. Distributed under a Creative Commons BY-SA License.



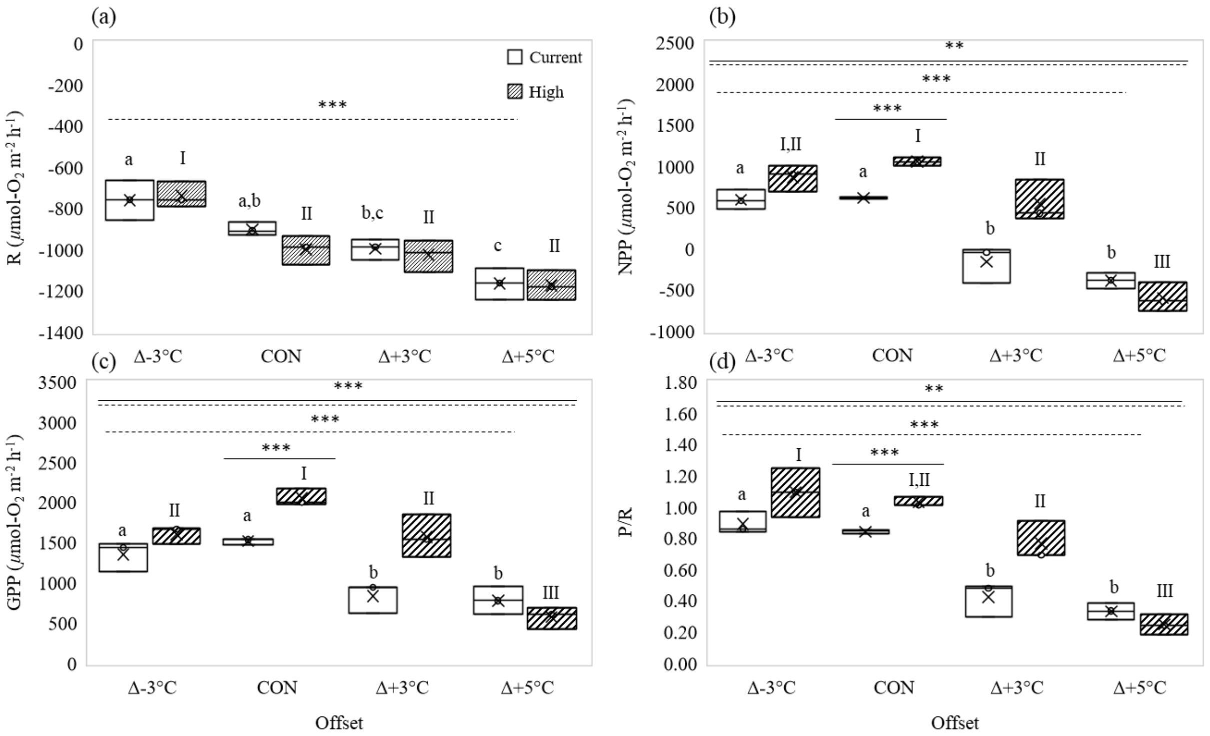
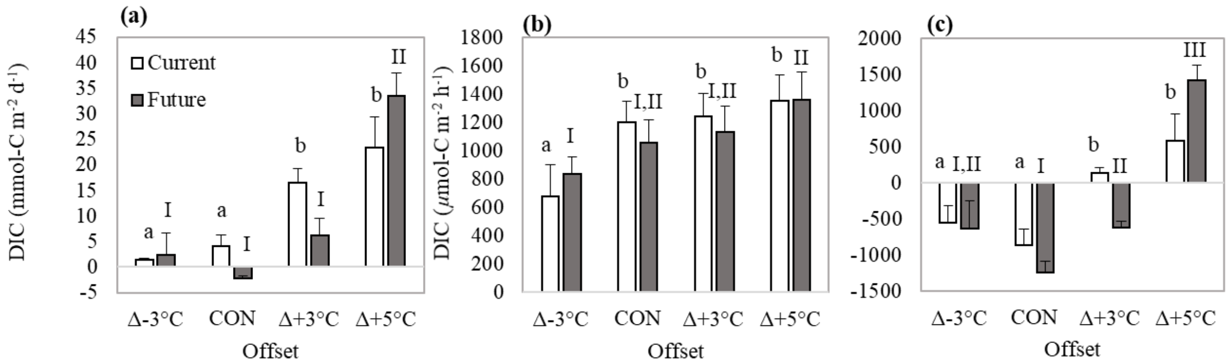


Figure 2. . Effect of temperature ~~(a) R~~on rates of (a) respiration, R, (b) net primary production, NPP~~ rates,~~, and (c) gross primary production, GPP ~~rates~~ (μmol-O$_2$ m^{-2} h^{-1}) and (d) P/R under current (open boxes) and ~~future~~high-*p*CO$_2$~~.~~ (hatched boxes). Panels show mean ~~(± SD)~~ values '×' at three temperature offsets from control (CON~~)~~, = 23 °C~~.~~). Middle horizontal line in each box is the

exclusive median, with the start of the upper and lower quartiles represented by the top and bottom edges of the box, respectively. Letters identify significantly different means across temperatures under current-*p*CO_2, and numerals identify significantly different means across temperatures under ~~future~~high-*p*CO_2 conditions~~. Letters~~, where letters or numerals that are the same indicate no significant difference, as determined by post hoc Tukey's test. Solid and dashed horizontal lines identify significant effects of OA and temperature, respectively, where double solid/dashed lines identify significant interaction of temperature and OA (two-way ANOVA). Levels of significance are denoted with '*' 0.05, '' 0.01, and '***' 0.001. Data in Table S3 and S4.**


(a)
DIC (mmol-C m-2 d-1)
Current
Future
Δ-3°C
CON
Δ+3°C
Δ+5°C
Offset
(b)
DIC (µmol-C m-2 h-1)
(c)
a
b
I
I,II
II
III

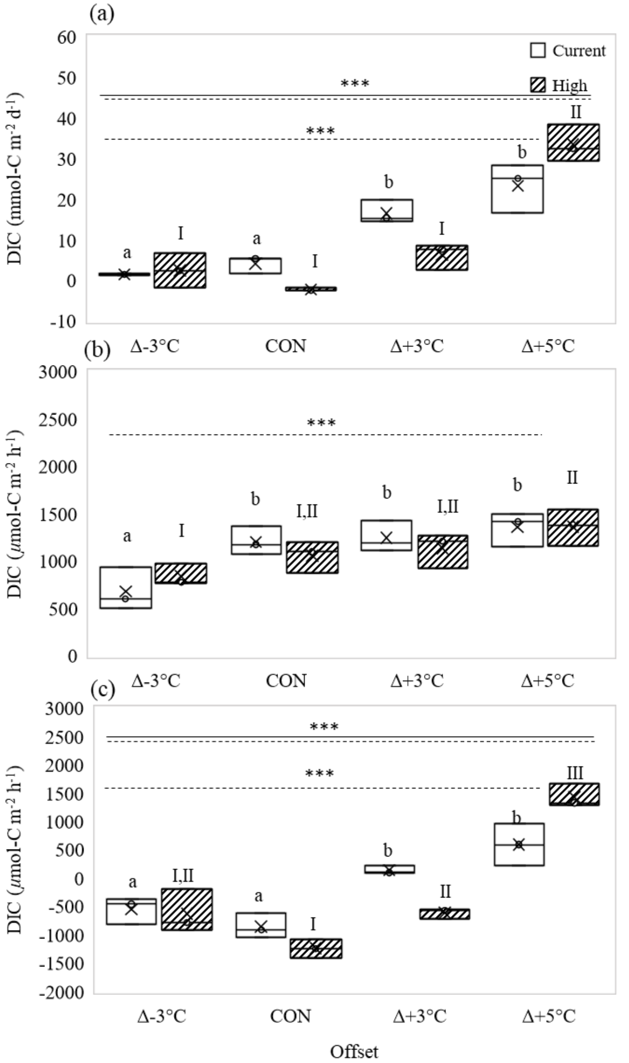
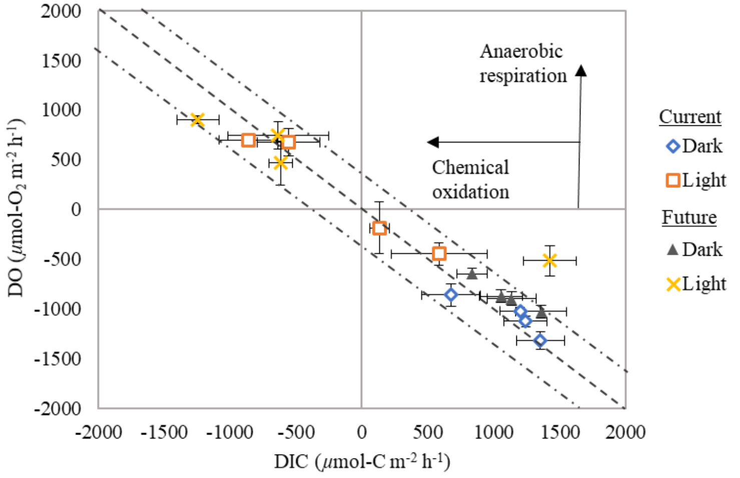

(a)
DIC (mmol-C m-2 d-1)
Current
High
***
(b)
DIC (µmol-C m-2 h-1)
(c)
DIC (µmol-C m-2 h-1)
Δ-3°C
CON
Δ+3°C
Δ+5°C
Offset
a
b
I
I,II
II
III

Figure 3. Effect of temperature on ~~DIC fluxes in the~~ (a) net ~~DIC~~dissolved inorganic carbon (DIC) production (mmol-C m^{-2} d^{-1}), and (b) dark and (c) light DIC fluxes (μmol-C m^{-2} h^{-1}) under current (open boxes) and ~~future~~high-pCO$_2$~~.~~ (hatched boxes). Panels show mean ~~(± SD) rates~~values 'X' at three temperature offsets from control conditions (CON~~),~~ = 23 °C~~.~~). Middle horizontal line in each box is the exclusive median, with the start of the upper and lower quartiles represented by the top and bottom edges of the box, respectively. Letters identify significantly different means across temperatures under current-pCO$_2$ and numerals identify significantly different means across temperatures under ~~future~~high-pCO$_2$ conditions~~. Letters~~, where letters or numerals that are the same indicate no significant difference, as determined by a one-way ANOVA and post hoc Tukey's test. Solid and dashed horizontal lines identify significant effects of OA and temperature, respectively, where double solid/dashed lines identify significant interaction of temperature and OA (two-way ANOVA). Levels of significance are denoted with '*' 0.05, '' 0.01, and '***' 0.001. Data in Table S5.**



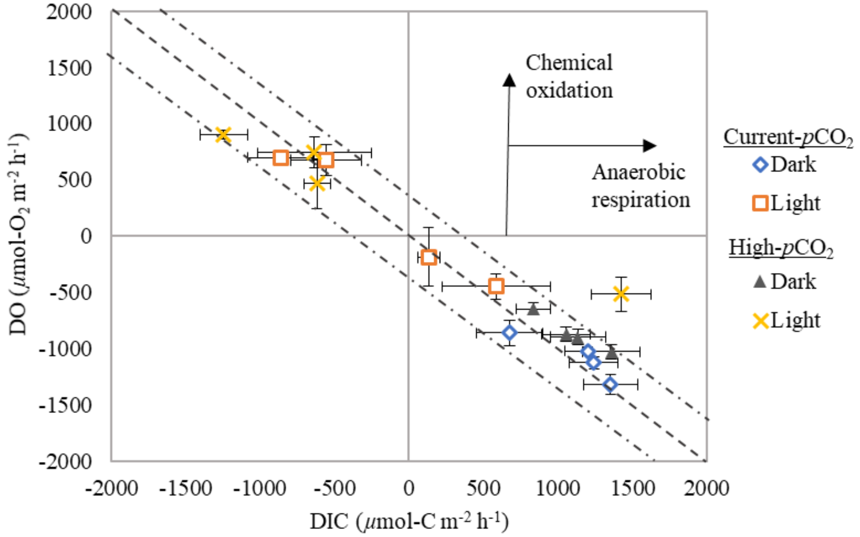
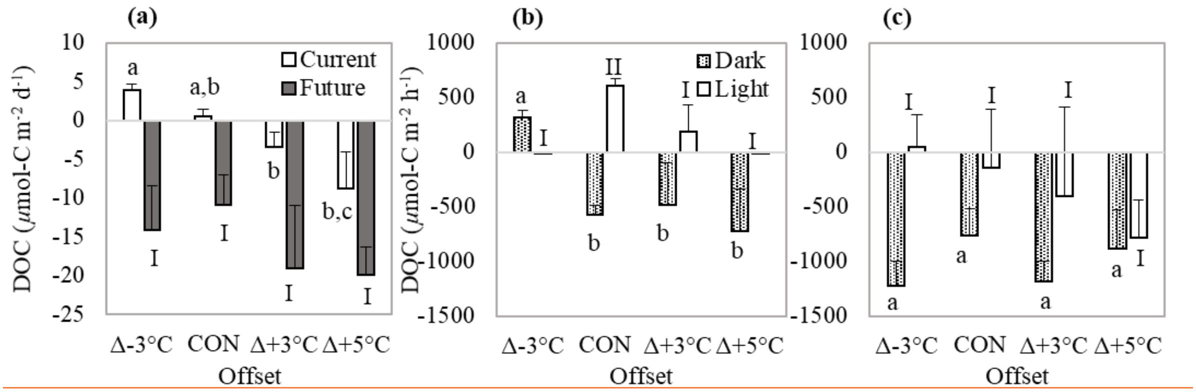


Figure 4. DIC:DO fluxes from sediment (μmol-C or -O$_2$ m^{-2} h^{-1}) for all temperatures in dark and light cycles subject to current and ~~future~~high-pCO$_2$ (mean ± SD) Dashed line highlights the 1:1 ratio (± 18%, Hopkinson, 1985) with values falling on this line likely a result of aerobic respiration. Arrows indicate the position values would fall in if sediments were experiencing chemical oxidation or anaerobic respiration.



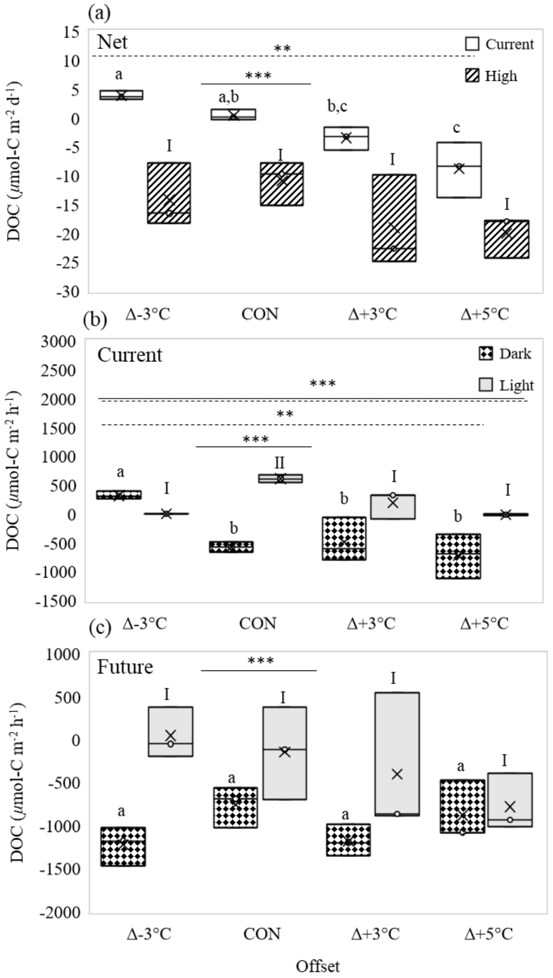


Figure 5. Effect of three temperature offsets from control (CON = 23°C) on ~~DOC fluxes under current and future pCO_2. Panel~~ (a) ~~shows mean (± SD)~~ net ~~DOC~~dissolved organic carbon (DOC) fluxes (μmol-C m^{-2} d^{-1}) under current ~~and future~~(open boxes) and high-

$p\mathrm{CO_2}$ (hatched boxes). Light (grey boxes) and dark fluxes (spotted boxes) of DOC (μmol-C m^{-2} h^{-1}) for (b) current-$p\mathrm{CO_2}$ and (c) high-$p\mathrm{CO_2}$ conditions ~~at three temperature offsets from control (CON), 23°C. Letters~~. In (a), letters identify significantly different means across temperatures under current-$p\mathrm{CO_2}$ and numerals identify significantly different means across temperatures under ~~future~~high-$p\mathrm{CO_2}$ conditions, where ~~letters or numerals that are the same indicate no significant difference. Light and dark fluxes for DOC (μmol-C m^{-2} h^{-1}) are presented~~ in ~~Panels~~ (b) and (c~~) for current-$p\mathrm{CO_2}$ and future-$p\mathrm{CO_2}$ conditions, respectively. Here,~~), letters identify significantly different means across temperatures in dark and numerals identify significantly different means across temperatures in light cycles. Letters or numerals that are the same indicate no significant difference~~.~~, as determined by a one-way ANOVA and post hoc Tukey's test. Solid and dashed horizontal lines identify significant effects of $p\mathrm{CO_2}$ or light and temperature, respectively, where double solid/dashed lines identify significant interaction of temperature and light (two-way ANOVA). Levels of significance are denoted with '*' 0.05, '' 0.01, and '***' 0.001. Data in Table S6.**

Supplementary tables available in "Supplement"

Table S4. Measured total alkalinity (TA) and DIC used to calculate pH (Free scale) using $\mathrm{CO_2}$SYS directly compared to the measured pH from the cores using HACH multiprobe meter with pH probe. Mean absolute difference was used to estimate uncertainty in $p\mathrm{CO_2}$ calculations via $\mathrm{CO_2}$SYS. Data used in a manuscript currently under review.

Table S5. Overlapping mean control rates (±SD) in current and ~~future~~high-$p\mathrm{CO_2}$ incubations for dark and light cycles. Units for dark and light rates (μmol-C or -$\mathrm{O_2}$ m^{-2} h^{-1}) and net rates (mmol-C or -$\mathrm{O_2}$ m^{-2} d^{-1}). Scaled means in Table S6 applied to significantly different means (*) only.

Table S6. Scaled means (± SD) for R and NPP rates (μmol-$\mathrm{O_2}$ m^{-2} h^{-1}) under current and ~~future~~high-$p\mathrm{CO_2}$ incubations. CON* is the overlapping control present both weeks (note: current control and CON current are the same).

Table S7. Gross primary productivity (GPP) and productivity to respiration ratio (P/R) calculated for each temperature under both current and high-$p\mathrm{CO_2}$.

Table S8. Dark and light fluxes of dissolved inorganic carbon (DIC) for each temperature under both current and high-$p\mathrm{CO_2}$.

Table S9. Dark and light fluxes dissolved organic carbon (DOC) for each temperature under both current and high-$p\mathrm{CO_2}$.

Table S7. DIN concentrations (μM) (mean ± SD) at the start (minimum) and end of the full incubation cycle.