

Interactive comment on “Warming enhances carbon dioxide and methane fluxes from Red Sea seagrass (*Halophila stipulacea*) sediments” by Celina Burkholz et al.

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Received and published: 28 October 2019

Authors' response

RC: Comments from referees/public, AR: Authors' response, AC: Authors' changes in manuscript

ANONYMOUS REFEREE #2

Review report of the paper entitled “Warming enhances carbon dioxide and methane fluxes from Red Sea seagrass (*Halophila stipulacea*) sediments” submitted by Celina Burkholz et al to Biogeosciences

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Received and published: 17 September 2019

1) RC: Seagrass meadows, saltmarshes and mangroves are the “hot spot” blue carbon sinks and highly efficient in long-term carbon storage in the coastal marine ecosystems. Deterioration of these natural marine ecosystems through anthropogenic perturbation could change their carbon sinks efficiency and may contribute to climate change through re-emissions of locked carbon dioxide and other greenhouse gases. The warming-dependent emissions of metabolic GHG in the coastal ecosystems are likely to vary for coastal sediment of different geological origin. It is expected that in situ temperature increase is particularly important in seagrass meadows which have high carbon sequestration with long-term storage capacity and trap organic matter from external sources. The extreme conditions in the Red Sea i.e. slower seagrass growth due to nutrient limitation and greater microbial degradation of soil organic carbon because of high temperature could be related to low Corg storage in the sediment compared to temperate meadows. This paper reports the results of the study on the response of sediment collected from two *H.stipulacea* meadows, S1 (22°56.775'N, 38°52.677'E) and S2 (22°54.742'N, 38°53.848'E) at Al Kharar, a lagoon on the Saudi coast of the central Red Sea in February-March 2018, in terms of air-seawater fluxes of CO₂ and CH₄ along with their isotopic signature from Red Sea meadows compared to for gradual increase of temperature from 25 °C to 37 °C and prolonged darkness. However, I had difficulties to understand certain sections of the manuscript including the methodology and discussion. Furthermore, the author did not address appropriately other important elements particularly the redox conditions, anthropogenic pressure in and around the the ecosystems (Seagrass and bare soil).

AR: We thank the reviewer for the thorough review and constructive comments. We have addressed the comments individually to revise and clarify any uncertainties.

2) RC: The sediment in the present study is of carbonate (82.61-91.75%) dominated. What constitutes the rest fraction (9-18%) of the 1It does not represent all types of sediment of different geological origin in Al Kharar lagoon. Previous reports (Serrano et

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al. 2018 Scientific Reports, 8:15037) indicate that soils in seagrass meadows in Saudi Arabia, Central Red Sea. are mainly constituted of clay and silt particles ($37 \pm 0.7\%$ on average), with a relatively high abundance of very fine sands ($21 \pm 0.4\%$) compared to fine sands ($16 \pm 0.4\%$), medium sands ($12 \pm 0.3\%$) and coarse sands ($14 \pm 0.7\%$). Youssef & El-Sorogy, 2016 (Arab J Geosci 9:474) showed that sediment textures consist of mud, gravelly sand, and sandy mud and organic matter in the sediment may also be derived from Mangroves which are common in Al-Kharrar lagoon between latitude $22^{\circ}45'$ and $23^{\circ}00'$ N and longitude $39^{\circ}00'$ and $38^{\circ}45'$ E.

AR: We agree with the reviewer that this study does not represent all types of different geological origin in Al Kharar. Our main focus was to see a difference between two different sites, and then focus on the difference between vegetated and bare cores. We have therefore made the decision not to include other sediment types. We have added some additional information to the discussion section regarding the origin of organic matter content.

AC: Page 10, line 4-13: The isotopic signature of the CO₂ released from bare sediments shifted with warming indicating a shift from seston (average $\delta^{13}\text{C}$ value of -25.43 ± 0.42 ‰ Duarte et al., 2018) as the organic matter supporting respiration to seagrass carbon (average $\delta^{13}\text{C}$ value of -7.73 ± 0.11 ‰ for Red Sea seagrass and -7.57 ± 0.15 ‰ for *H. stipulacea* in the Red Sea; Duarte et al., 2018) as the source of CO₂. The mean $\delta^{13}\text{C}$ value of Red Sea seagrass sediments was reported to be -13.36 ± 0.4 (Garcias-Bonet et al., 2019a), similar to the results found in this study. In the vegetated cores, the isotopic composition of CO₂ stayed rather constant, indicating seagrass to be the main organic carbon source regardless of warming. Similar results were found in a recent study applying stable isotope mixing models, with the major contributors to the organic matter in seagrass sediments in the Red Sea being seagrass leaves and macroalgae blades, with contributions of 43 and 37 %, respectively (Garcias-Bonet et al., 2019a). We also observed a shift to a lighter isotopic signature of CH₄ with warming, thereby

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indicating an increasing CH₄ production by methanogens with warming (Whiticar, 1990).

3) RC: Why you have collected sediment from two *H. stipulacea* meadows only?

AR: We thank the reviewer for the comment. Only two meadows were chosen for this hypothesis as the main focus was to test the effect of warming. Al Kharar is an enclosed lagoon where *H. stipulacea* forms dense patches and can be found growing along an OM gradient. We have chosen these two meadows to test our hypothesis that there is a difference along an OM gradient. Both meadows are monospecific, while many other meadows in Al Kharar are comprised of different seagrass species.

4) RC: Why the sediment of two *H. stipulacea* meadows showed high bulk density (1.1 - 1.28 g cm⁻³) relative to silt-clay sediment (1.05 g cm⁻³) in seagrass meadows in Saudi Arabia, Central Red Sea Serrano et al. 2018 observed. The bulk density of carbonate sediment (carbonate 0.7 g/cm³) should be even lower than that of sediment containing mainly sand (1.586 g cm⁻³) and claysilt (1.0 to 1.6 g/cm³). Organic carbon content of both vegetated (0.43 - 0.55%) and bare (0.41 - 0.52%) carbonate sediment of *H. stipulacea* meadows is greater than the value reported for clay-silty sediment of other seagrass meadows (0.33% , Serrano et al. 2018).

AR: We thank the reviewer for pointing this out. Unfortunately, we don't have more data on sediment composition and grain size to confirm the other parts of the sediment composition. We acknowledge that the sediment composition differed from that reported in Serrano et al. (which includes Professor Carlos M. Duarte, under whose supervision both that and this research was conducted). We do not expect the sediments we sampled to be representative of all sediment configurations found in seagrass meadows in the Red Sea, nor Serrano et al. (2018) designed their research to encompass all sediment types occurring across the Red Sea.

5) RC: Why the production of *H. stipulacea* is high at the study site S2 compared to S1 and other seagrass meadows along the Saudi coast even though there was almost no

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available nutrients (N & P), and no difference in nutrients between S1 and S2.

AR: We thank the reviewer for their concern. S2 had a higher organic matter content and a higher seagrass biomass compared to S1, which could have affected the fluxes. Additionally, previous studies have generally found a high variability in fluxes. We have rearranged this section to improve clarity.

AC: Page 8, line 20-page 10, line: 10: 4.1 Carbon dioxide and methane air-seawater fluxes The values reported for CO₂ and CH₄ fluxes varied greatly between the two sites studied here, with higher fluxes in the more organic sediments with higher biomass (S2). CO₂ and CH₄ fluxes were also highly variable over time in the studied site, as the first evaluation of fluxes in the same location delivered rates up to 100-fold above the rates of the second measurement one week later. Hence, organic matter availability along with temperature may account for the large variation in CO₂ and CH₄ fluxes. Additionally, the variability of CO₂ and CH₄ fluxes could also be supported by infaunal species present in the cores that were not recorded in this study. These trends were similar to results reported in previous studies, as a high variability between species and locations was found (cf. Table 1 in Garcias-Bonet and Duarte (2017)). Even though there were some differences, carbon, nitrogen and phosphorus concentrations were generally similar, and they didn't seem to have an effect on CO₂ and CH₄ fluxes. Carbon, nitrogen and phosphorus concentrations were low compared to mean values (Carbon: 33.6 ± 0.31 % DW, nitrogen: 1.92 ± 0.05 % DW, phosphorus: 0.23 ± 0.011 % DW) reported for seagrass leaves by Duarte (1990). Serrano et al. (2018) explained the discrepancy between Red Sea data and global data with the extreme conditions in the Red Sea, such as low nutrient input and high temperatures, as well as a limited data set favoring high carbon stocks in the Mediterranean. The results presented here add to those by Garcias-Bonet and Duarte (2017) to identify Red Sea seagrass communities as a significant source of CH₄. The presence of seagrass resulted in a higher organic matter supply to the sediments, favoring the presence of methanogens, which led to higher CH₄ fluxes compared to those fluxes supported in bare sediments (Barber

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and Carlson, 1993; Bahlmann et al., 2015), consistent with the up to 100-fold higher CH₄ fluxes supported by vegetated compared to bare sediments in this study. Additionally, higher fluxes in vegetated cores could be an indicator of direct effects resulting from the presence of seagrass, as vascular plants on land have shown to have varying effects on methane emissions caused by differences in biomass and gross photosynthesis (Öquist and Svensson, 2002). Similar trends were also seen by Garcias-Bonet and Duarte (2017) who reported an increase in CH₄ fluxes with increasing organic matter content in Red Sea seagrass sediments. They reported organic matter contents in Red Sea seagrass sediments ranging from 2.33 ± 0.07 % (*Halodule uninervis*) to 12.42 ± 0.23 % (*Enhalus acoroides*), including a mixed meadow with *H. stipulacea* and *H. uninervis* showing a slightly higher organic matter content of 3.51 ± 0.17 % compared to vegetated sediments at S2. Moreover, they found the highest CH₄ fluxes in meadows with the highest biomass, confirming our findings with higher fluxes in study site S2. In terms of CO₂ equivalent greenhouse potential, only the bare sediment maintained at 25 °C seemed to act as a C sink over the experimental period, while the vegetated sediments, both maintained at 25 °C and exposed to warming, acted as sources of greenhouse gases. A sublethal disturbance, such as warming below the lethal threshold, can therefore lead to a shift of seagrass ecosystems from acting as net sinks to net sources of greenhouse gases, as demonstrated experimentally here.

6) RC: If seagrass detritus is the significant source of soil organic carbon, what is the source of organic carbon for bare sediment? Why vegetated and bare sediment of S1 showed no difference in soil organic carbon content?

AR: We thank the reviewer for their concern. Al Kharar is an enclosed lagoon receiving organic matter inputs from mangroves, macroalgae, phytoplankton, as well as from land occasionally. We have added some additional information to the discussion section

AC: Page 10, line 4-13: The isotopic signature of the CO₂ released from bare sediments shifted with warming indicat-

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ing a shift from seston (average $\delta^{13}\text{C}$ value of -25.43 ± 0.42 ‰ Duarte et al., 2018) as the organic matter supporting respiration to seagrass carbon (average $\delta^{13}\text{C}$ value of -7.73 ± 0.11 ‰ for Red Sea seagrass and -7.57 ± 0.15 ‰ for *H. stipulacea* in the Red Sea; Duarte et al., 2018) as the source of CO_2 . The mean $\delta^{13}\text{C}$ value of Red Sea seagrass sediments was reported to be -13.36 ± 0.4 (Garcias-Bonet et al., 2019a), similar to the results found in this study. In the vegetated cores, the isotopic composition of CO_2 stayed rather constant, indicating seagrass to be the main organic carbon source regardless of warming. Similar results were found in a recent study applying stable isotope mixing models, with the major contributors to the organic matter in seagrass sediments in the Red Sea being seagrass leaves and macroalgae blades, with contributions of 43 and 37 %, respectively (Garcias-Bonet et al., 2019a). We also observed a shift to a lighter isotopic signature of CH_4 with warming, thereby indicating an increasing CH_4 production by methanogens with warming (Whiticar, 1990).

7) RC: The $\delta^{13}\text{C}$ -Corg showed depletion of ^{13}C in comparison to leaf and both vegetated and bare sediment showed no significant difference of $\delta^{13}\text{C}$ -Corg. Moreover, given $\delta^{13}\text{C}$ value of -7.96 ± 0.27 ‰ is lower than that of literature value for C_4 plants -14 ‰. This needs clarifications. Use stable isotope mixing model to determine the actual contribution of seagrass on organic carbon content of the meadow sediment. CO_2 fluxes were also 10-fold higher in vegetated compared to adjacent bare sediments, indicating elevated microbial remineralization rates in vegetated sediments.

AR: We share the reviewer's concern regarding the contributors to the organic matter in the sediment. Unfortunately, we cannot perform such analysis for the study site as we have not sampled all possible contributors for $\delta^{13}\text{C}$ analysis as it was out of the scope of this study. However, we are now discussing our results on the light of a comprehensive assessment of sources of organic carbon to sediments in Red Sea seagrass meadows we published since (Garcias-Bonet et al. (2019).

AC: Page 10, line 4-13: The isotopic signature of the CO_2
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released from bare sediments shifted with warming indicating a shift from seston (average $\delta^{13}\text{C}$ value of -25.43 ± 0.42 ‰ Duarte et al., 2018) as the organic matter supporting respiration to seagrass carbon (average $\delta^{13}\text{C}$ value of -7.73 ± 0.11 ‰ for Red Sea seagrass and -7.57 ± 0.15 ‰ for *H. stipulacea* in the Red Sea; Duarte et al., 2018) as the source of CO_2 . The mean $\delta^{13}\text{C}$ value of Red Sea seagrass sediments was reported to be -13.36 ± 0.4 (Garcias-Bonet et al., 2019a), similar to the results found in this study. In the vegetated cores, the isotopic composition of CO_2 stayed rather constant, indicating seagrass to be the main organic carbon source regardless of warming. Similar results were found in a recent study applying stable isotope mixing models, with the major contributors to the organic matter in seagrass sediments in the Red Sea being seagrass leaves and macroalgae blades, with contributions of 43 and 37 %, respectively (Garcias-Bonet et al., 2019a). We also observed a shift to a lighter isotopic signature of CH_4 with warming, thereby indicating an increasing CH_4 production by methanogens with warming (Whiticar, 1990).

8) RC: P 4: Before measuring fluxes, why the water inside the cores was replaced by fresh seawater ? Is it accumulated pore water ? Replacement of pore water saturated with CO_2 and CH_4 by fresh sea water may result serious error!

AR: We thank the reviewer for this comment as we realized the text was not clear enough. We did not replace the pore water, we replaced the water overlying the sediment inside the cylindrical core, in order to be sure that measurements started with the same initial concentrations. We have changed the text to avoid confusion.

AC: Page 4, line 19-22: Before measuring fluxes, the water overlying the sediment inside the cores was carefully siphoned until only 5 mm of water remained over the sediment surface and fresh seawater was carefully siphoned in the core, to avoid disturbing the redoxcline, leaving a headspace of approx. 5 - 6 cm, and the cores were closed again with stoppers containing gas tight valves.

9) RC: Sediment core was incubated at one particular temperature and CO₂ and CH₄ concentrations were measured in 10 ml of head space air samples from each core at 0, 12hrs (light) and 12 hrs (dark). Considering, efluxes of 10,422 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ d}^{-1}$ and 88.11 $\mu\text{mol CH}_4 \text{ m}^{-2} \text{ d}^{-1}$, simple calculation shows after 12 hrs increased amount of CO₂ and CH₄ should be 37 μmol and 0.31 μmol , respectively. But standards (A: 750 ppm CO₂, 9.7 ppm CH₄, B: 250.5 ppm CO₂, 3.25 ppm CH₄) used before each (?) measurement of samples are all above those values. A plot of observed mixing ratio of CO₂ and CH₄ versus temperature should be given for better understanding.

AR: We thank the reviewer for their concern. We reported rates in this study, meaning that we calculated the difference between the first measurement (T₀) and the 3rd measurement after 24 hours (T₂). The actual measurements of the headspace air sample in ppm are not reported in this study. To avoid confusion, we have deleted the sentences referring to the light and dark fluxes, as we only report the daily fluxes. We changed the method section accordingly:

AC: Page 4, line 27-28: The daily CO₂ and CH₄ fluxes were calculated from the difference between T₀ and T₂ taking into account the core surface area ($\mu\text{mol m}^{-2} \text{ d}^{-1}$).

Page 5, line 34-page 6, line 2: The daily CO₂ fluxes were calculated from the difference between T₀ and T₂ taking into account the core surface area ($\mu\text{mol m}^{-2} \text{ d}^{-1}$). Daily CH₄ fluxes were estimated using the same calculations as for the CO₂ fluxes with the exception of the Bunsen solubility coefficient.

10) RC: The mean ratio of CH₄/CO₂ was found to be 0.008 which seems to be higher than the previous value reported for seagrass meadows (*Halodule uninervis*, *Halodule pinifolia*, *Halophila ovalis*, *Halophila ovata*, and *Halophila beccarii*), Chilika Lagoon (PLoS ONE 13(10): e0203922. <https://doi.org/10.1371/journal.pone.0203922>).

AR: We thank the reviewer for pointing this out. We've added additional information to the result and discussion section.

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AC: Page 7, line 31: The CH₄/CO₂ ratio declined in the vegetated sediments exposed to warming from 7 to 0.8 %. Page 10, line 1-3: Increasing water temperature led to a decrease in the CH₄/CO₂ ratio. While there was ~7 % of sequestered carbon released as CH₄ to the atmosphere in vegetated sediments at 25 °C (on day 2), it decreased to ~0.8 % in vegetated sediments at 37 °C. In contrast, Banerjee et al. (2018) reported ~1% of carbon being released as CH₄.

11) RC: The organic matter content was higher in S2 (vegetated 0.55%) than in S2 (bare 0.52%) by 5% . Is it below the error limit of its determination?

AR: We thank the reviewer for the comment. We found slightly higher organic matter content in S2 compared to S, in both vegetated and bare sediments, and a t-test was used to determine significance. We have changed this section accordingly.

AC: Page 6, line 32-33: The organic matter content was slightly higher in S2 than in S1, in both vegetated (t-test, $p < 0.0001$) and bare (t-test, $p < 0.001$) sediments (Table 1).

12) RC: How do you explain 6-fold CO₂ and 100-fold CH₄ greater emission in S2 (vegetated) than in S2 (bare) ? Provide a plot of observed CH₄ concentration versus temperature. AR: We thank the reviewer for pointing this out. A 6-fold higher CO₂ flux was reported for vegetated compared to bare sediments in both S1 and S2 indicating the difference between vegetated and bare cores. The same trend was seen for CH₄ fluxes with up to 100-fold higher fluxes in vegetated compared to bare sediments. A plot of observed CO₂ and CH₄ concentrations vs temperature is shown in Fig. 2. The second x-axis indicates the experimental temperature for the community exposed to warming from 25 - 37 °C. We added some additional information to the discussion section.

AC: Page 8, line 21-40: The values reported for CO₂ and CH₄ fluxes varied greatly between the two sites studied here, with higher fluxes in the more organic sediments with higher biomass (S2). CO₂ and CH₄ fluxes were also highly variable over time in the studied site, as the first evaluation of fluxes in the same location delivered rates

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up to 100-fold above the rates of the second measurement one week later. Hence, organic matter availability along with temperature may account for the large variation in CO₂ and CH₄ fluxes. Additionally, the variability of CO₂ and CH₄ fluxes could also be supported by infaunal species present in the cores that were not recorded in this study. These trends were similar to results reported in previous studies, as a high variability between species and locations was found (cf. Table 1 in Garcias-Bonet and Duarte (2017)). Even though there were some differences, carbon, nitrogen and phosphorus concentrations were generally similar, and they didn't seem to have an effect on CO₂ and CH₄ fluxes. Carbon, nitrogen and phosphorus concentrations were low compared to mean values (Carbon: 33.6 ± 0.31 % DW, nitrogen: 1.92 ± 0.05 % DW, phosphorus: 0.23 ± 0.011 % DW) reported for seagrass leaves by Duarte (1990). Serrano et al. (2018) explains the discrepancy between Red Sea data and global data with the extreme conditions in the Red Sea, such as low nutrient input and high temperatures, as well as a limited data set favoring high carbon stocks in the Mediterranean. The results presented here add to those by Garcias-Bonet and Duarte (2017) to identify Red Sea seagrass communities as a significant source of CH₄. The presence of seagrass resulted in a higher organic matter supply to the sediments, favoring the presence of methanogens, which led to higher CH₄ fluxes compared to those fluxes supported in bare sediments (Barber and Carlson, 1993; Bahlmann et al., 2015), consistent with the up to 100-fold higher CH₄ fluxes supported by vegetated compared to bare sediments in this study. Additionally, higher fluxes in vegetated cores could be an indicator of direct effects resulting from the presence of seagrass, as vascular plants on land have shown to have varying effects on methane emissions caused by differences in biomass and gross photosynthesis (Öquist and Svensson, 2002).

13) RC: The Fig 2 D and E shows large scatter of data and drawing those straight lines have no use.

AR: We thank the reviewer for their comment. Fig 2 D and E show a decline in CH₄ fluxes over time when the sediments were maintained at 25 °C, both in vegetated

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($R^2 = 0.43$, $p < 0.001$, Fig. 2D) and, less strongly, bare sediments ($R^2 = 0.24$, $p < 0.01$; Fig. 2E, Table S2). Lines represent a fitted linear model. We have added the following sentence to the figure heading for clarification:

AC: Page 17, line 7: Lines represent a fitted linear regression equation.

14) RC: The CH₄ fluxes declined over time when the sediments were maintained at 25 °C, both in vegetated (Fig. 2D) and, less strongly, bare sediments. In contrast, CH₄ fluxes tended to increase with temperature in vegetated (Fig. 2D) and bare (Fig. 2E) sediments gradually warmed from 25 °C to 37 °C, although it was not significant. Since the in situ redox condition of both water and sediment was not maintained during experiment that may affect the equilibrium between counteracting microbial processes of production and oxidation in the sediment. The study on the response of benthic net methane concentrations to higher temperatures needs also to take into account methane production rates, Q₁₀ values, and community sizes of methanogens and methanotrophs in seagrass sediments. I believe that the manuscript needs significant revision before being considered for resubmission.

AR: We thank the reviewer for the thorough review and constructive comments. We have addressed the comments individually, and we have revised the discussion section accordingly.

Specific comments

Abstract:

15) RC: In the first sentence please mention if Seagrasses are net source of CO₂ and CH₄.

AR: We thank the reviewer for their comment, the first sentence mentions that seagrasses can be both, sources and sinks of CO₂ and CH₄. We have changed the sentence for better understanding.

AC: Page 1, line 9-10: Seagrass meadows are autotrophic ecosystems acting as car-

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bon sinks, but they have also been shown to be sources of carbon dioxide (CO₂) and methane (CH₄).

16) RC: Line 17: "We detected distinct differences between vegetated and unvegetated sediments, with the vegetated sediments supporting 6-fold higher CO₂ fluxes, and 10- to 100-fold higher CH₄ fluxes" This is a confusing statement. What are the conditions for the high flux was not mentioned?

AR: We thank the reviewer for pointing this out. The sentence only relates to the difference between vegetated and bare sediment, not different conditions. We changed the sentence for clarification.

AC: Page 1, line 17-18: We detected 6-fold higher CO₂ fluxes in vegetated compared to bare sediments, as well as 10- to 100-fold higher CH₄ fluxes.

Methodology

17) RC: "Assessment of carbon dioxide and methane air-seawater fluxes" This section is not clear. Please clarify the total number of samples collected from each core.

AR: We thank the reviewer for their comment. Three samples were taken from each core. The following sentence was changed for clarification: AC: Page 4, line 26-27: One sample from each core was taken at the start (T₀), after 12 hours of light (T₁) and after 12 hours of dark (T₂). 18) RC: Was there a periodic collection?

AR: When referring to the collection of cores, cores were collected in February (comparison S1 and S2), March (temperature) and May (darkness) 2018. Samples from each core were collected taken at the start (T₀), after 12 hours of light (T₁) and after 12 hours of dark (T₂). After the 24 hours measuring period, cores had time to acclimate to the new temperature before another measurement period happened.

19) RC: Did the cores have seagrass in the top surface? What was the depth of the core sample?

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AR: We thank the reviewer for pointing out the missing information. Yes, there was seagrass in the vegetated sediments. We added the depth to the text for clarification.

AC: Page 3, line 18-20: Two *H. stipulacea* meadows at a depth of 2-3 m, S1 (22°56.775'N, 38°52.677'E) and S2 (22°54.742'N, 38°53.848'E), were chosen to represent a range 20 of organic matter content in the sediment, selected to evaluate greenhouse gas fluxes.

20) RC: What were the criteria for fixing the light condition at 70 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ at different incubation conditions?

AR: 70 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ was the setting in the incubator chambers where the samples stayed only during the measurements, while the cores were exposed to 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in the aquaria between sampling days.

21) RC: "The temperature in the second aquarium was increased at a rate of 1 $^{\circ}\text{C day}^{-1}$." Why this was done? Is this a natural increase (with 1 day time) for the physiological adaptation by of seagrass?

AR: We thank the reviewer for pointing out the missing information. A temperature increase of 1 $^{\circ}\text{C d}^{-1}$ was chosen to allow the seagrass to adjust to the higher temperature instead of creating stress by raising the temperature abruptly. We have added the missing information.

AC: Page 4, line 37-38: The temperature in the second aquarium was increased at a rate of 1 $^{\circ}\text{C day}^{-1}$ to allow for acclimatization of the vegetated and bare cores.

22) RC: I wonder how they have measured water-air flux from a system enclosed with sediment- water-air.

AR: We thank the reviewer for his/her comment. We followed the headspace technique as described in Garcias-Bonet et al. (2017) and Sea et al. (2018) that allows to measure air-sea fluxes: The water inside the cores overlying the sediment was replaced by fresh seawater leaving a headspace, and the cores were closed again with stoppers

C14

containing gas tight valves. The cores were then left for one hour to allow for equilibration between the seawater and the headspace air. 10 mL of air were sampled from each core using a syringe, and injected the air sample in a cavity ring-down spectrometer through a small sample isotopic module extension, which provided both the partial pressure and the isotopic carbon composition of the CO₂ and CH₄ in the air sample.

23) RC: Lots of Q10 values are available in the literature. Do those values agree with the present report?

AR: We thank the reviewer for his/her comment. Q10 values in the literature indicate that respiration rates in seagrass have a higher temperature dependence compared to photosynthesis, while methanogenesis has a higher thermal dependence compared to photosynthesis and respiration. Our results agree with these findings as we saw an increase in CO₂ and CH₄ fluxes with warming. We added some additional information to the result and the discussion section:

AC: Page 7, line 31-33: For CO₂ and CH₄ fluxes in vegetated sediments, the Q10 value for the temperature range 25-37 °C was 9 and 1.5, respectively, while the Q10 value for bare sediments was 13.8 and 4.2, respectively.

Page 9, line 32-33: Additionally, previous research has shown that methanogenesis has a higher thermal dependence than respiration and photosynthesis (Yvon-Durocher et al., 2014) confirming the trends seen here with increasing fluxes at higher temperatures.

Results:

24) RC: The first sentence needs to be revised.

AR: We thank the reviewer for pointing this out. We have changed the sentence for clarification.

AC: Page 6, line 26-27: Carbon, nitrogen (N), and phosphorus (P) concentrations in seagrass leaves were low, but they were 4- to 40-fold higher than vegetated and bare

C15

sediment concentrations (Table 1).

25) RC: Page 6 line 28: "There were no consistent differences in C, N and concentration in bare and vegetated sediments (Table 1)." Does it indicate limited influence of additional carbon storage in the seagrass sediment? "The mean C, N content is significantly lower in the seagrass leaf (Table 1) compared to global average carbon content of 35% (Duarte 1990; Fourqurean et al. 2012).

AR: We thank the reviewer for his/her concern. Serrano et al. (2018) also reported low organic carbon content for Red Sea seagrass sediments due to the extreme conditions in the Red Sea (high temperatures and low nutrient input).

AC: Page 8, line 29-34: Even though there were some differences, carbon, nitrogen and phosphorus concentrations were generally similar, and they didn't seem to have an effect on CO₂ and CH₄ fluxes. Carbon, nitrogen and phosphorus concentrations were low compared to mean values (Carbon: 33.6 ± 0.31 % DW, nitrogen: 1.92 ± 0.05 % DW, phosphorus: 0.23 ± 0.011 % DW) reported for seagrass leaves by Duarte (1990). Serrano et al. (2018) explains the discrepancy between Red Sea data and global data with the extreme conditions in the Red Sea, such as low nutrient input and high temperatures, as well as a limited data set favoring high carbon stocks in the Mediterranean.

26) RC: There are several other seagrass sps apart from *Halophila* sp. in Kharar lagoon with considerable spatial variation sediment composition. Those Sps should also directly or indirectly affect the sediment composition. The sediment in the present study is mainly composed of coral sand. Is it possible for the occurrence of such high concentration of OM in the sediment with no clay and silt?

AR: *Halophila* sp. is by far the dominant species in Al Kharar lagoon, forming a large monospecific meadow. Mixed meadows do occur, but the contribution of species other than *Halophila* is anecdotal at the ecosystem level. We did not measure the grain size distribution of the sediments sampled, so we have no basis to suggest that there was

C16

no clay or silt, as the reviewer infers (but we did not suggest such thing).

27) RC: Page 6 Line 38: "The daily CO₂ flux was up to 6-fold higher in vegetated compared to bare sediments, and tended to be generally higher in S2 compared to S1, where bare sediments showed net CO₂ uptake, although differences were not significant" This result need to be discussed in details in the discussion section.

AR: We thank the reviewer for pointing this out. We rearranged the discussion section accordingly.

AC: Page 8, line 20-page 9, line 10: 4.1 Carbon dioxide and methane air-seawater fluxes The values reported for CO₂ and CH₄ fluxes varied greatly between the two sites studied here, with higher fluxes in the more organic sediments with higher biomass (S2). CO₂ and CH₄ fluxes were also highly variable over time in the studied site, as the first evaluation of fluxes in the same location delivered rates up to 100-fold above the rates of the second measurement one week later. Hence, organic matter availability along with temperature may account for the large variation in CO₂ and CH₄ fluxes. Additionally, the variability of CO₂ and CH₄ fluxes could also be supported by infaunal species present in the cores that were not recorded in this study. These trends were similar to results reported in previous studies, as a high variability between species and locations was found (cf. Table 1 in Garcias-Bonet and Duarte (2017)). Even though there were some differences, carbon, nitrogen and phosphorus concentrations were generally similar, and they didn't seem to have an effect on CO₂ and CH₄ fluxes. Carbon, nitrogen and phosphorus concentrations were low compared to mean values (Carbon: 33.6 ± 0.31 % DW, nitrogen: 1.92 ± 0.05 % DW, phosphorus: 0.23 ± 0.011 % DW) reported for seagrass leaves by Duarte (1990). Serrano et al. (2018) explains the discrepancy between Red Sea data and global data with the extreme conditions in the Red Sea, such as low nutrient input and high temperatures, as well as a limited data set favoring high carbon stocks in the Mediterranean. The results presented here add to those by Garcias-Bonet and Duarte (2017) to identify Red Sea seagrass communities as a significant source of CH₄. The presence of seagrass resulted in a higher organic

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matter supply to the sediments, favoring the presence of methanogens, which led to higher CH₄ fluxes compared to those fluxes supported in bare sediments (Barber and Carlson, 1993; Bahlmann et al., 2015), consistent with the up to 100-fold higher CH₄ fluxes supported by vegetated compared to bare sediments in this study. Additionally, higher fluxes in vegetated cores could be an indicator of direct effects resulting from the presence of seagrass, as vascular plants on land have shown to have varying effects on methane emissions caused by differences in biomass and gross photosynthesis (Öquist and Svensson, 2002). Similar trends were also seen by Garcias-Bonet and Duarte (2017) who reported an increase in CH₄ fluxes with increasing organic matter content in Red Sea seagrass sediments. They reported organic matter contents in Red Sea seagrass sediments ranging from 2.33 ± 0.07 % (*Halodule uninervis*) to 12.42 ± 0.23 % (*Enhalus acoroides*), including a mixed meadow with *H. stipulacea* and *H. uninervis* showing a slightly higher organic matter content of 3.51 ± 0.17 % compared to vegetated sediments at S2. Moreover, they found the highest CH₄ fluxes in meadows with the highest biomass, confirming our findings with higher fluxes in study site S2. In terms of CO₂ equivalent greenhouse potential, only the bare sediment maintained at 25 °C seemed to act as a C sink over the experimental period, while the vegetated sediments, both maintained at 25 °C and exposed to warming, acted as sources of greenhouse gases. A sublethal disturbance, such as warming below the lethal threshold, can therefore lead to a shift of seagrass ecosystems from acting as net sinks to net sources of greenhouse gases, as demonstrated experimentally here.

28) RC: Page 7 Line 21: "Despite CO₂ and CH₄ fluxes showing the same response to warming in both types of sediment, vegetated sediments held higher fluxes than bare sediments. The relationship between net CO₂ and CH₄ fluxes in bare vs. vegetated sediments showed that both bare and vegetated communities tended to act as net CO₂ sinks at 25 °C, but tended to act as CO₂ sources at warmer temperatures (Fig. 3A), whereas net CH₄ fluxes were 3- to 8-fold higher in vegetated compared to bare sediments. (Fig. 3B)." The organic carbon contents are comparable for seagrass and bare soil. Do the result indicate higher susceptibility of the seagrass soil carbon at a

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higher temperature?

AR: We agree with the reviewer that this could be an indication for higher susceptibility of seagrass sediment to higher temperatures. We have added some additional information in the discussion section.

AC: Page 9, line 13-15: Both CO₂ and CH₄ fluxes were higher in vegetated compared to adjacent bare sediments, indicating elevated remineralization rates in vegetated sediments as well as a higher susceptibility of seagrass sediment to increasing temperatures.

29) RC: Apart from regulation by Seagrass, CH₄ and CO₂ efflux depend on the redoxcline which has not been highlighted in relation to the abundance of methanogens and methanotrops. In methodology of the incubation experiment it is mentioned that “. . .the water inside the cores was replaced by fresh seawater from the aquaria leaving a headspace of approx. 5 - 6 cm. . .”. Did this replacement change the redox condition of surface water?

AR: We thank the reviewer for his/her concern, which is due to insufficient detail in our account of procedures. This replacement was done gently to avoid disturbing the redoxcline. The replacement of the water was done through carefully siphoning the water in the core, retaining 5 mm of water overlaying the corer to avoid disturbing the redoxcline. Fresh seawater was then also carefully siphoned into the corer. Changing the water overlying the sediment allowed us to start with similar values at the first sampling (T₀) to then see how the CO₂ and CH₄ fluxes were affected over a 24 hours period. Since we used the air from the headspace to determine air-water fluxes, we did not highlight the redoxcline in the water column. The replacement with freshwater would not change the redoxcline relative to that present in the environment, whereas leaving the water that was trapped inside the corers closed and locked could lead to accumulation of products released from the sediment that do not accumulate in the natural environment, where water flows freely and air-sea exchange operates. Hence,

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removing the water that was contained in closed corers and replacing it with fresh seawater is a standard procedure in sediment core incubations (e.g. Foster and Fullweiller 2019, for a recent paper), necessary to avoid, rather than introduce, artifacts. We now specify how this replacement was done.

AC: Page 4, line: 19-22: Before measuring fluxes, the water overlying the sediment inside the cores was carefully siphoned until only 5 mm of water remained over the sediment surface and fresh seawater was carefully siphoned in the core, to avoid disturbing the redoxcline, leaving a headspace of approx. 5 - 6 cm, and the cores were closed again with stoppers containing gas tight valves.

Reference: Foster, S.Q. and Fulweiler, R.W., 2019. Estuarine Sediments Exhibit Dynamic and Variable Biogeochemical Responses to Hypoxia. *Journal of Geophysical Research: Biogeosciences*, 124(4), pp.737-758.

Discussion:

30) RC: This section is inadequate and the observed results need more detailed discussions about the variations CO₂ and CH₄ fluxes at natural, high temperature and low light conditions. “Similar trends were seen by Garcias-Bonet and Duarte (2017) who reported an increase in CH₄ fluxes with increasing organic matter content in Red Sea seagrass sediments.” In the present study lower CO₂ and CH₄ fluxes were recorded from adjacent bare sediments with comparable Organic C content.

AR: We thank the reviewer for his/her comment. We have changed the discussion according to the comments. We based this statement on the fact that the vegetated sediment of S2 had a higher OM content than bare sediments of S2, as well as vegetated and bare sediments in S1. Concurrently, these were the cores with the highest fluxes.

AC: Page 8, line 21-page 9, line 10: The values reported for CO₂ and CH₄ fluxes varied greatly between the two sites studied here, with higher fluxes in the more organic

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sediments with higher biomass (S2). CO₂ and CH₄ fluxes were also highly variable over time in the studied site, as the first evaluation of fluxes in the same location delivered rates up to 100-fold above the rates of the second measurement one week later. Hence, organic matter availability along with temperature may account for the large variation in CO₂ and CH₄ fluxes. Additionally, the variability of CO₂ and CH₄ fluxes could also be supported by infaunal species present in the cores that were not recorded in this study. These trends were similar to results reported in previous studies, as a high variability between species and locations was found (cf. Table 1 in Garcias-Bonet and Duarte (2017)). Even though there were some differences, carbon, nitrogen and phosphorus concentrations were generally similar, and they didn't seem to have an effect on CO₂ and CH₄ fluxes. Carbon, nitrogen and phosphorus concentrations were low compared to mean values (Carbon: 33.6 ± 0.31 % DW, nitrogen: 1.92 ± 0.05 % DW, phosphorus: 0.23 ± 0.011 % DW) reported for seagrass leaves by Duarte (1990). Serrano et al. (2018) explains the discrepancy between Red Sea data and global data with the extreme conditions in the Red Sea, such as low nutrient input and high temperatures, as well as a limited data set favoring high carbon stocks in the Mediterranean. The results presented here add to those by Garcias-Bonet and Duarte (2017) to identify Red Sea seagrass communities as a significant source of CH₄. The presence of seagrass resulted in a higher organic matter supply to the sediments, favoring the presence of methanogens, which led to higher CH₄ fluxes compared to those fluxes supported in bare sediments (Barber and Carlson, 1993; Bahlmann et al., 2015), consistent with the up to 100-fold higher CH₄ fluxes supported by vegetated compared to bare sediments in this study. Additionally, higher fluxes in vegetated cores could be an indicator of direct effects resulting from the presence of seagrass, as vascular plants on land have shown to have varying effects on methane emissions caused by differences in biomass and gross photosynthesis (Öquist and Svensson, 2002). Similar trends were also seen by Garcias-Bonet and Duarte (2017) who reported an increase in CH₄ fluxes with increasing organic matter content in Red Sea seagrass sediments. They reported organic matter contents in Red Sea seagrass sediments ranging from

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2.33 ± 0.07 % (*Halodule uninervis*) to 12.42 ± 0.23 % (*Enhalus acoroides*), including a mixed meadow with *H. stipulacea* and *H. uninervis* showing a slightly higher organic matter content of 3.51 ± 0.17 % compared to vegetated sediments at S2. Moreover, they found the highest CH₄ fluxes in meadows with the highest biomass, confirming our findings with higher fluxes in study site S2.

Page 9, line 18-23: However, the fluxes maintained at 25 °C showed a net CO₂ uptake with a mean of 464.78 ± 156.6 μmol CO₂ m⁻² d⁻¹ (Table S1), while those reported in a mixed *Halodule* sp. and *Halophila* sp. meadow in India showed a net CO₂ release (dry season: $1,190 \pm 1,600$ μmol CO₂ m⁻² d⁻¹, wet season: $18,400 \pm 8,800$ μmol CO₂ m⁻² d⁻¹; Banerjee et al., 2018). Both values reported were measured at higher temperatures (dry season: 30 ± 0.68 °C, wet season: 27.94 ± 0.72 °C, Banerjee et al., 2018) compared to our fluxes measured at 25 °C, also indicating that temperature might lead to higher fluxes.

Page 9, line 26-31: Mean CH₄ fluxes at in situ temperature (25 °C) in vegetated sediments were lower than the mean value of 85.1 ± 27.8 μmol CH₄ m⁻² d⁻¹ reported for other seagrass meadows in the Red Sea (Garcias-Bonet and Duarte, 2017). In contrast, the community exposed to warming reached a maximum average CH₄ flux almost 4-fold higher than the community held at 25 °C, and showed a clear increase with warming, relative to sediments held at 25 °C. The increase in CH₄ fluxes with warming was consistent with reports from Barber and Carlson (1993) for a *Thalassia testudinum* community in Florida Bay and Garcias-Bonet and Duarte (2017) for Red Sea seagrass communities, who reported higher CH₄ production rates at higher temperatures.

31) RC: Page 8 Line 24: ". . .while the CO₂ fluxes in vegetated sediments maintained at 25 °C decreased over time." Why it was high initially and decreased gradually? explain.

AR: We thank the reviewer for the comment. The initial higher value might have been

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a response to the stress due to sample collection and transportation. Even though we allowed the cores time to adapt, this value could still be an indicator for the experienced disturbance. All values measured after more than 5 days of sampling showed negative values indicating the capacity of seagrass sediments to act as carbon sinks. We added the following information to the discussion section.

AC: Page 9, line 23-25: An initial high CO₂ flux measured on day 2 after sampling could be an indicator for the experienced disturbance due to sample collection and transportation even though we allowed the cores some time to adapt.

32) RC: Line 30: "The presence of seagrass resulted in a higher organic matter supply to the sediments, favoring the presence of methanogens, which led to higher CH₄ fluxes compared to those fluxes supported in bare sediments (Barber and Carlson, 1993; Bahlmann et al., 2015), consistent with the up to 100-fold higher CH₄ fluxes supported by vegetated compared to bare sediments in this study." In the present study, soil organic C content in vegetated and adjacent bare sediment are comparable but CH₄ fluxes are significantly different. Does the results (high emissions at S1 and S2) indicated direct CH₄ emission from Seagrass? (pls see: Quist and Svensson, 2002, Vascular plants as regulators of methane emissions from a subarctic mire ecosystem, *Jgr*, 107, NO. D21, 4580 and others) Effect of warming

AR: We thank the reviewer for the comment. We have rearranged the discussion section accordingly and added some additional information:

AC: Page 8, line 34-40: The results presented here add to those by Garcias-Bonet and Duarte (2017) to identify Red Sea seagrass communities as a significant source of CH₄. The presence of seagrass resulted in a higher organic matter supply to the sediments, favoring the presence of methanogens, which led to higher CH₄ fluxes compared to those fluxes supported in bare sediments (Barber and Carlson, 1993; Bahlmann et al., 2015), consistent with the up to 100-fold higher CH₄ fluxes supported by vegetated compared to bare sediments in this study. Additionally, higher fluxes in

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vegetated cores could be an indicator of direct effects resulting from the presence of seagrass, as vascular plants on land have shown to have varying effects on methane emissions caused by differences in biomass and gross photosynthesis (Öquist and Svensson, 2002).

33) RC: Page 8, Line 26 : "However, the fluxes maintained at 25 °C were about 3-fold below those reported in a mixed *Halodule* sp. and *Halophila* sp. meadow in India (980 μmol CO₂ m⁻² d⁻¹; Banerjee et al., 2018)." Is this the mean flux (the present value) or the least value recorded or the values represent during the end of the incubation at 25 °C? In the previous sentence it was mentioned as "CO₂ fluxes in vegetated sediments maintained at 25 °C decreased over time"

AR: We thank the reviewer for pointing out this error. We have edited the sentence accordingly.

AC: Page 9, line 18-23: However, the fluxes maintained at 25 °C showed a net CO₂ uptake with a mean of 464.78 ± 156.6 μmol CO₂ m⁻² d⁻¹ (Table S1), while those reported in a mixed *Halodule* sp. and *Halophila* sp. meadow in India showed a net CO₂ release (dry season: 1,190 ± 1,600 μmol CO₂ m⁻² d⁻¹, wet season: 18,400 ± 8,800 μmol CO₂ m⁻² d⁻¹; Banerjee et al., 2018). Both values reported were measured at higher temperatures (dry season: 30 ± 0.68 °C, wet season: 27.94 ± 0.72 °C, Banerjee et al., 2018) compared to our fluxes measured at 25 °C, also indicating that temperature might lead to higher fluxes.

34) RC: Page 8, Line 40: "We also reported a 10-fold decline in CH₄ fluxes over time for sediment communities maintained at 25 °C, which could be attributable to increased sulfate reduction, reduced CH₄ production or a combination of both. Methane is produced under anoxic conditions in marine sediments, yet only a small portion is released, as CH₄ production by methanogens is compensated for by CH₄ consumption by sulfatereducing bacteria (Barnes and Goldberg, 1976)." The soil redox conditions were not measured in this study. Please show some evidence of enhanced soil anox-

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icity which may have increased SO₄ reduction. What could be the other reason/s of reduced CH₄ production.

AR: We thank the reviewer for the comment. We have added more information to this paragraph to improve clarity and address the reviewer's concern.

AC: Page 9, line 34-40: We also reported a 10-fold decline in CH₄ fluxes over time for sediment communities maintained at 25 °C, which could be attributable to increased sulfate reduction, reduced CH₄ production or a combination of both. Methane is produced under anoxic conditions in marine sediments, yet only a small portion is released, as CH₄ production by methanogens is compensated for by CH₄ oxidation by sulfate-reducing bacteria (Barnes and Goldberg, 1976). Similar to the trends seen in CO₂ fluxes, the decrease in CH₄ fluxes could be attributable to an initial stress response to the disturbance caused by sample collection and transportation. While reduced photosynthetic activity and a decrease in biomass could result in higher CH₄ fluxes (Lyimo et al., 2018), the cores maintained at 25 °C might show the effect of healthy conditions.

Conclusion:

35) RC: This section may be rewritten after revising the discussion section with few more synthesizing statements.

AR: We thank the reviewer for the comment, we have changed the conclusion accordingly.

AC: Page 11, line 3-13: In summary, this study reports, for the first time, experimental evidence that warming leads to increased greenhouse gas (CO₂ and CH₄) fluxes in a *H. stipulacea* meadow in the Red Sea, and it may lead to seagrass meadows shifting from acting as sinks to sources of greenhouse gases. Increased fluxes at higher temperatures can be an indication of higher remineralization rates and a higher susceptibility of vegetated sediments to temperature. The elevated organic matter content, higher

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biomass and higher plant activity in vegetated sediments led to increased CO₂ and CH₄ fluxes in vegetated compared to bare sediments and a much steeper increase in CO₂ and CH₄ fluxes with warming. In addition, prolonged darkness led to an increase in CO₂ fluxes, while CH₄ fluxes decreased over time, also indicating organic matter to be the driver. However, we also found a high variability in fluxes over time indicating that other factors, such as infaunal species, could play a role as well. While current focus is on conserving blue carbon ecosystems from losses due to deteriorated water quality or mechanical damage, our results show that sublethal warming may also lead to emissions of greenhouse gases from seagrass meadows, contributing to a feedback between ocean warming and further climate change.

36) RC: Figure 2: what does the blue dots represent?

AR: We thank the reviewer for pointing out the missing information, the blue dots represent "Constant temperature, 25 °C". We have added the missing information to the graph on page 17.

37) RC: Figure 3: . . . the dashed line indicates line 1:1, and dotted lines show lines 2:1, 4:1 and 8:1. Need to be detailed.

AR: We thank the reviewer for pointing this out. We used the additional lines to visualize the relationship between vegetated and bare sediments showing that CH₄ fluxes were 3- to 8-fold higher in vegetated compared to bare sediments.

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2019-93>, 2019.

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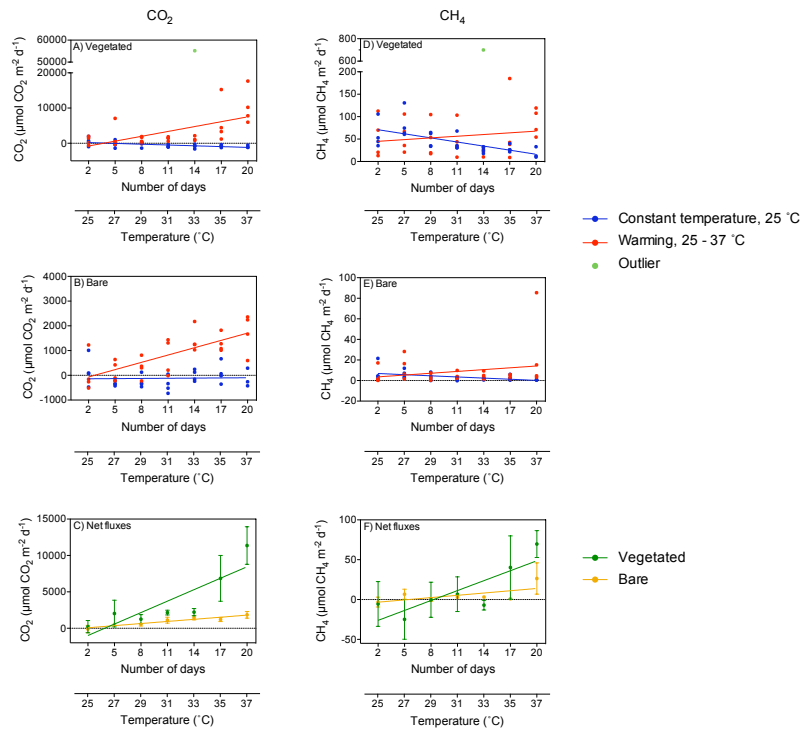


Fig. 1. Fig. 2