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Warming enhances carbon dioxide and methane fluxes from Red Sea seagrass (Halophila stipulacea) sediments

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Abstract. Seagrass meadows are autotrophic ecosystems storing carbon in their biomass and sediments, but they have also been shown to be sources of carbon dioxide (CO_2) and methane (CH_4). Seagrasses can be negatively affected by increasing seawater temperatures, but the effects of warming on CO_2 and CH_4 fluxes in seagrass meadows have not yet been reported. Here, we examine the effect of two disturbances on air-seawater fluxes of CO_2 and CH_4 in Red Sea *Halophila stipulacea* communities compared to adjacent unvegetated sediments using cavity ring-down spectroscopy. We first characterized CO_2 and CH_4 fluxes in vegetated and adjacent unvegetated sediments, and then experimentally examined their response, along with that of the C isotopic signature of CO_2 and CH_4, to gradual warming from 25 ˚C (winter seawater temperature) to 37 ˚C, 2 ˚C above current maximum temperature. In addition, we assessed the response to prolonged darkness, thereby providing insights into the possible role of suppressing plant photosynthesis in supporting CO_2 and CH_4 fluxes. We detected distinct differences between vegetated and unvegetated sediments, with the vegetated sediments supporting 6-fold higher CO_2 fluxes, and 10- to 100-fold higher CH_4 fluxes. Warming led to an increase in net CO_2 and CH_4 fluxes, reaching average fluxes of 10,422.18 ± 2,570.12 µmol CO_2 m^{-2} d^{-1} and 88.11 ± 15.19 µmol CH_4 m^{-2} d^{-1}, while CO_2 and CH_4 fluxes decreased over time in sediments maintained at 25 ˚C. Prolonged darkness led to an increase in CO_2 fluxes but a decrease in CH_4 fluxes in vegetated sediments. These results add to previous research identifying Red Sea seagrass meadows as a significant source of CH_4, while also indicating that sublethal warming may lead to increased emissions of greenhouse gases from seagrass meadows, providing a feedback mechanism that may contribute to further enhance global warming.

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1 Introduction

Global warming, as a result of anthropogenic emissions of greenhouse gases, has led to ocean warming by 0.11 ˚C between 1971 to 2010 (0 - 75 m, IPCC, 2014) with the global mean sea-surface temperature predicted to increase further with additional emissions, depending on emission scenarios (IPCC, 2014). Ocean warming is leading to a shift in species and ecosystem processes (Hoegh-Guldberg and Bruno, 2010), including metabolic processes that are under strong thermal control (Brown et al., 2004; Garcias-Bonet et al., 2018, 2019).

Ecosystem metabolism can also be a source of greenhouse gases, depending on the metabolic balance of the community, where autotrophic communities [net community production (NCP) > respiration (R)] act as a sink for carbon dioxide (CO_2), while heterotrophic communities [net community production (NCP) < respiration (R)] act as a source of CO_2 (Duarte et al., 2011). Since respiration rates tend to increase faster with warming than primary production does (Brown et al., 2004; Harris et al., 2006; Regaudie-De-Gioux and Duarte, 2012), warming may lead to typically autotrophic ecosystems, such as seagrass meadows, shifting to net heterotrophic, thereby switching from acting as sinks to sources of CO_2 (Harris et al., 2006). Emissions of metabolic greenhouse gases with ocean warming may provide a feedback mechanism by which anthropogenic emissions of greenhouse gases may lead to warming-dependent emissions by coastal ecosystems, therefore enhancing climate warming. This feedback effect is particularly likely to occur where methane (CH_4) is released, as CH_4 is calculated to have a global warming potential 28 times larger than CO_2 per mol of carbon C emitted (Myhre et al., 2013).

Indeed, CO_2 and CH_4 emissions from some tropical mangrove forests have been calculated to partially offset the capacity of mangroves to act as C sinks (Rosentreter et al., 2018). Whereas the emission of CO_2 and CH_4 from seagrass ecosystems has received far less attention, seagrass ecosystems have been reported to support CH_4 emissions of the order of 1.4 to 401.3 µmol CH_4 m^{-2} d^{-1} (cf. Table 1 in Garcias-Bonet and Duarte (2017)). Provided estimates of their global extent of seagrass meadows ranging from a documented 326,000 km^2 (Unsworth et al., 2018) to a predicted 1.6 million km^2 (Jayathilake and Costello, 2018), seagrass meadows may be important, yet hitherto overlooked contributors to CH_4 emissions. Garcias-Bonet and Duarte (2017) reported that seagrasses could contribute to global CH_4 emissions by releasing CH_4 at a rate of 0.09 - 2.7 Tg yr^{-1}, which may increase the contribution of marine global emissions to previously reported global estimates by about 30 % (Garcias-Bonet and Duarte, 2017).

Seagrasses are known to be autotrophic ecosystems, acting as C sinks (Duarte et al., 2010) supporting a global burial rate of 27.4 Tg C yr^{-1} (Duarte et al., 2005). They store carbon in their below- and above-ground biomass on a short term, as well as in their sediment on a long-term (Duarte et al., 2005). They account for 10 % of the C storage in ocean sediments even though they only cover 0.2 % of the ocean surface (Duarte et al., 2005; Fourqurean et al., 2012). However, disturbances can lead to the loss of biomass and the emissions of stored C turning blue carbon ecosystems into C sources (Macreadie et al., 2015; Lovelock et al., 2017; Arias-Ortiz et al., 2018) which will ultimately contribute to global emissions intensifying the greenhouse effect. Lyimo et al. (2018) showed that stressors such as shading and grazing led to an increase of CH_4 emissions by seagrass ecosystems by reducing their photosynthetic capacity. Garcias-Bonet and Duarte (2017) reported that CH_4 release from seagrass sediments tended to increase with seawater temperature, and suggested that CH_4 emissions by seagrass ecosystems may be under temperature control in the Red Sea. Indeed, some seagrass ecosystems in the Red Sea have shown to shift from an autotrophic to a heterotrophic state during the warmer summer months, indicating that some seagrass communities might already grow past their thermal optimum (Burkholz et al., 2019a).

The Red Sea ranks as the warmest sea in the world, with summer seawater temperatures reaching 35 ˚C, and is warming at higher rates than those of the global ocean (Chaidez et al., 2017). Provided respiration rates and also CH_4 fluxes in seagrass ecosystems are likely to increase with temperature, seagrass meadows in the Red Sea may be close to shifting from net sinks to net sources of greenhouse gases with further warming. Emission rates are also dependent on organic carbon supply, as high sediment organic matter can promote an increase in CH_4 production (Sotomayor et al., 1994; Gonsalves et al., 2011) and organic matter released from seagrass photosynthesis may also stimulate CO_2 and CH_4 production in the sediment community. Indeed, sediments in seagrass ecosystems support a 1.7-fold higher organic matter content than surrounding bare sediments, not only due to the slow turn-over of biomass but also due to their ability to trap particles (Kennedy et al., 2010; Duarte et al., 2013).

Here, we test the hypothesis that CO_2 and CH_4 fluxes by seagrass communities increase with warming. We do so by experimentally examine the effect of warming and plant activity on air-seawater fluxes of CO_2 and CH_4 in a Red Sea seagrass (*Halophila stipulacea)* community. The tropical seagrass species *Halophila stipulacea* (Forsskål) Ascherson is native to the Indian Ocean and is one of the most common species in the Red Sea (Qurban et al., 2019). It seems to be highly adaptive to various environments, as it is now found as an exotic species in the Mediterranean (Lipkin, 1975) and the Caribbean Sea (Ruiz and Ballantine, 2004), indicating its high resilience to changing conditions (Por, 1971). We first characterize air-seawater fluxes of CO_2 and CH_4 in Red Sea *Halophila stipulacea* communities compared to adjacent unvegetated sediments, and then experimentally examine their response, along with that of the C isotopic signature of CO_2 and CH_4, to gradual warming from 25 ˚C to 37 ˚C. In addition, we assess the response to prolonged darkness, thereby providing insights into the possible role of plant photosynthesis in supporting CO_2 and CH_4 fluxes.

2 Material and methods

2.1 Study site and sample collection

Samples were collected at Al Kharar, a lagoon on the Saudi coast of the central Red Sea in February 2018. Two *H. stipulacea* meadows, 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 of organic matter content in the sediment, selected to evaluate greenhouse gas fluxes. Moreover, the *H. stipulacea* meadow in the middle of the lagoon (S2) with higher biomass density (Table 1) was chosen as the study site to experimentally assess the role of temperature and darkness in greenhouse gas fluxes. The seagrass and sediment community was sampled using translucent cylindrical PVC cores (26 cm length and 9.5 cm in diameter). The sharpened edge of the core was carefully pushed approximately 10 cm into the sediment with a rubber hammer so that the structure of leaves, roots and sediment stayed intact. A rubber stopper was then placed on top, before the core was carefully pulled out of the sediment without disturbing the structure and another rubber stopper was placed on the bottom of the core. The sediment cores were immediately transported to the laboratory.

2.2 Sediment and plant characterization

Once the cores were opened, the first 10 cm of the sediment and the plant biomass were collected and dried at 60 ˚C to a constant dry weight. To characterize the two different *H. stipulacea* meadows, sediment and plant biomass samples were then ground to analyze the sediment composition and conduct nutrient analyses. A 50 mL tube was filled with sediment from the first 10 cm and the contents dried at 60 ˚C to a constant dry weight and weighed to determine the sediment bulk density (g sediment cm^{-3}). Organic matter content was analyzed by loss on ignition (LOI, Dean, 1974). Approx. 5 g of dried sediment were placed in a muffle furnace and burned at 450 ˚C for 5 hours. The organic matter content was calculated as:

$$\%OM = \frac{pre-ignition\ weight(g) - post-ignition\ weight\ (g)}{pre-ignition\ weight\ (g)} \times 100 \qquad (1)$$

The carbonate content was estimated using a Pressure Gauge Calcimeter. Approx. 1 g of sample was placed in the calcimeter and the recipient was filled with 10 % hydrochloric acid (HCl). The mass of $CaCO_3$ in the sample (g) was then calculated as follows:

$$m_{CaCO_3} = \frac{p - b}{a \times w} \qquad (2)$$

where *p* is the pressure recorded (ppm), *b* is the slope and *a* the intercept derived from the calibration curve, and *w* is the exact weight of each sediment sample (g). The percentage of $CaCO_3$ in the sample (% DW) was then calculated using Eq. 3:

$$\%_{CaCO_3} = \frac{m_{CaCO_3}}{w \times 100} \qquad (3)$$

Dried sediment and plant samples were digested using USEPA method 3052 and analyzed with nitric acid (HNO_3) and HCl using USEPA method 200.7 following manufacturer instructions. The phosphorus content (% DW) was analyzed using inductively coupled plasma optical emission spectroscropy (ICP-OES) on an Agilent 5110 ICP-OES (Agilent Technologies, Santa Clara, CA, USA). The C and N concentration of both plants and sediments was analyzed after acidification with HCl (Hedges and Stern, 1984), using Flash 2000 Organic Elemental Analyzer (CHNS/O-2, Thermo Fisher Scientic, Waltham, MA, USA). The isotopic signature of ^{13}C in sediment organic matter was analyzed, using cavity ring-down spectroscropy (CRDS G2201-I, Picarro Inc., Santa Clara, CA, USA), from the ^{13}C of CO_2 released by a combustion module (Costech Analytical Technologies Inc., CA, USA) delivering the CO_2 resulting from combusting the sediment organic matter to the CRDS instrument.

2.3 Assessment of carbon dioxide and methane air-seawater fluxes

In February 2018, triplicate cores from vegetated and adjacent bare (about 5 m from the edge of the seagrass patch) sediments were collected from sites S1 and S2 and transferred to incubation chambers (Percival Scientific Inc., Perry, IA, USA) set at 25 ˚C and a 12 hours light (up to 70 µmol photons m^{-2} s^{-1}) : 12 hours dark (12 h L : 12 h D) cycle to measure the greenhouse gas (CO_2 and CH_4) fluxes supported by these communities. Before measuring fluxes, the water inside the cores was replaced by fresh seawater from the aquaria leaving a headspace of approx. 5 - 6 cm, and the cores were closed again with stoppers containing gas tight valves. The cores were left for one hour to allow for equilibration between the seawater and the headspace air. We then sampled 10 mL of air from each core using a syringe, and injected the air sample in a cavity ring-down spectrometer (CRDS; Picarro Inc., Santa Clara, CA, USA) through a small sample isotopic module extension (SSIM A0314, Picarro Inc., Santa Clara, CA, USA), which provided both the partial pressure and the isotopic carbon composition of the CO_2 and CH_4 in the air sample. Samples were taken at the start (T0), after 12 hours of light (T1) and after 12 hours of dark (T2). Before and after each sampling, two standards were measured (A: 750 ppm CO_2, 9.7 ppm CH_4, B: 250.5 ppm CO_2, 3.25 ppm CH_4).

2.4 Effect of warming on carbon dioxide and methane air-seawater fluxes

In March 2018, we collected eight vegetated and eight bare sediment cores from site S2 to evaluate the response of greenhouse gas fluxes to warming. The sampling was performed as described above. The cores were transferred to the Coastal and Marine Resources Core Lab (CMOR, KAUST, Saudi Arabia). Nine vegetated and nine bare sediment cores were placed in each two aquaria with flow-through seawater set at *in situ* temperature (25 ˚C) and a 12 h L (up to 200 µmol photons m^{-2} s^{-1}): 12 h D cycle. One aquarium was maintained at 25 ˚C over the entire duration of the experiment to serve as a control for temperature-independent variability in fluxes. The temperature in the second aquarium was increased at a rate of 1 ˚C day^{-1}. CO_2 and CH_4 fluxes were measured at every 2 ˚C from 25 - 37 ˚C, with parallel measurements conducted on the cores maintained at 25 ˚C. After a one day acclimation period at each new temperature, the cores were closed with the stoppers and transferred to incubation chambers (Percival Scientific Inc., Perry, IA, USA) set at the target temperature for CO_2 and CH_4 flux measurements as described above. After the 24h measurements, the cores were returned to the aquaria. An additional core kept at each of the constant temperature and warming sets was sampled every four days (i.e. at 4 ˚C temperature intervals in the warming treatment) to analyze sediment composition. The cores used for fluxes estimates were opened after the final measurement (20 days since collection) to estimate the plant biomass, analyze the sediment composition at the end of the experiment.

2.5 Effect of darkness on carbon dioxide and methane air-seawater fluxes

In May 2018, six vegetated and six bare sediment cores were collected from site S2 and kept at a constant 25 ˚C with a 24 hours dark cycle. Only during the measurements in the incubators, the cores were exposed to a 12 h L : 12 h D cycle, allowing to compare fluxes with those measured in cores permanently maintained under the 12 h L : 12 h D photoperiod. CO_2 and CH_4 fluxes were measured after the first day of acclimation and then kept in the aquaria until signs of seagrass mortality started to become apparent, which occurred after one week in the dark. CO_2 and CH_4 fluxes were measured at alternate days as detailed above. At the end of the experiment (21 days since collection), the cores were opened and sampled to assess plant biomass, sediment composition.

2.6 Measurements of carbon dioxide and methane air-seawater fluxes

The concentration of CO_2 in the seawater after equilibrium was calculated based on the concentration of CO_2 in the headspace (ppm) measured by CRDS according to Sea et al. (2018) and Wilson et al. (2012):

$$[CO_2]_w = 10^{-6}\beta m_a p_{dry} \qquad (4)$$

where β is the Bunsen solubility coefficient of CO_2 (mol mL^{-1} atm^{-1}), m_a is the CO_2 concentration measured in the headspace (ppm), and p_{dry} is the atmospheric pressure of dry air (atm). The Bunsen solubility coefficient of CO_2 was calculated using Eq. 5:

$$\beta = H^{cp} \times (RT) \qquad (5)$$

where H^{cp} is the Henry constant (mol mL^{-1} atm^{-1}) calculated using the *marelac* R package (Soetaert et al., 2010). R is the ideal gas constant (0.082057459 atm L mol mL^{-1} K^{-1}) and T is the standard temperature (273.15 K).

The atmospheric pressure of dry air (p_{dry}) was calculated as follows:

$$p_{dry} = p_{wet}(1 - \%H_2O) \qquad (6)$$

where p_{wet} is the atmospheric pressure of wet air. The Boyle's law was applied as gas was collected several times from the same core.

The concentration of dissolved CO_2 in seawater before equilibrium was then calculated using Eq. 7:

$$[CO_2]_{aq} = \frac{[CO_2]_w V_w + 10^{-6} m_a V_a}{V_w} \qquad (7)$$

where V_w is the volume of seawater (mL) and V_a is the volume of the headspace (mL). The units were then converted to nM:

$$[CO_2]_{aq} = \frac{10^9 \times p_{dry}[CO_2]_{aq}}{RT} \qquad (8)$$

The daily CO_2 fluxes were calculated from the difference between T0 and T2 taking into account the core surface area (µmol m^{-2} d^{-1}). The light and dark fluxes were calculated from the change between CO_2 concentration after 12 hours of light and 12 hours of dark treatment, respectively, and were also transformed to an aerial basis (µmol m^{-2} h^{-1}).

Daily and hourly CH_4 fluxes were estimated using the same calculations as for the CO_2 fluxes with the exception of the Bunsen solubility coefficient. The Bunsen solubility coefficient was calculated as a function of the seawater temperature and salinity following Wiesenburg and Guinasso (1979). The total CO_2 greenhouse-equivalent fluxes were calculated assuming CH_4 to have a greenhouse potential 28-fold greater than that of CO_2 per mol of C (Myhre et al., 2013).

2.7 Isotopic composition of carbon dioxide ($\delta^{13}C$-CO_2) and methane ($\delta^{13}C$-CH_4)

The isotopic signature of CO_2 and CH_4 produced during the incubations was estimated using Keeling plots following Garcias-Bonet and Duarte (2017). $\delta^{13}C$ of CO_2 and CH_4 produced in our incubations was extracted from the intercept of the linear regression between the inverse of the gas concentration (ppm^{-1}) and the isotopic signature measured from the discrete samples in the CDRS instrument.

2.8 Data analysis

The data was analyzed for normality using the Shapiro-Wilk test. Mann-Whitney and t-test were used to test for differences in seagrass and sediment composition between sites and between vegetated and bare sediments, and ANOVA and Kruskal-Wallis test were used to test for differences between vegetated and bare sediments and both sites. To assess differences in greenhouse gas fluxes between different *H. stipulacea* communities, differences in CO_2 and CH_4 fluxes were analyzed between sites and between vegetated and bare sediment by using Kruskal-Wallis test. Trends in the flux between the communities experiencing warming and the ones maintained at 25 ˚C, as well as in the isotopic signature of $\delta^{13}C$-CO_2 and $\delta^{13}C$-CH_4 over time were analyzed by linear regression. When assessing the effect of darkness on greenhouse gas fluxes, the trend of CO_2 and CH_4 fluxes and their isotopic signatures were analyzed by linear regression. The statistical analyses were conducted in PRISM 5 (GraphPad Software, La Jolla, CA, USA) and JMP Pro 13.1.0 (SAS Institute Inc., Cary, NC, USA). The data is openly available from Burkholz et al. (2019b).

3 Results

3.1 Seagrass and sediment composition

Carbon, nitrogen, and phosphorus concentrations in seagrass leaves were low, but C, N and P leaf concentrations were 4- to 40-fold higher than sediment concentrations (Table 1). Seagrass sampled in site S1 had the highest C, N and P concentrations in the leaves, while sediment C and P concentrations differed significantly between sites (ANOVA, $p < 0.05$ and Kruskal-Wallis, $p < 0.001$, respectively), with the highest C and the lowest N concentrations found in the sediment of S2 (Table 1). There were no consistent differences in C, N and concentration in bare and vegetated sediments (Table 1).

The sediments had high, but variable, carbonate concentrations, which differed between sites (Kruskal-Wallis, $p < 0.0001$; Table 1). The organic matter content was higher in S2 than in S1, in both vegetated (t-test, $p < 0.0001$) and bare (t-test, $p < 0.001$) sediments (Table 1). Sediment bulk density was similar in both S1 and S2 sites, but vegetated sediments in S1 showed significantly lower bulk density compared to bare sediments (t-test, $p < 0.05$; Table 1). Seagrass biomass was significantly higher in S2 compared to S1 (t-test, $p < 0.05$). The isotopic signature of sediment organic carbon ranged across sites from -15.77 ± 0.07 ‰, in vegetated sediments, to -16.36 ± 0.28 ‰, in bare sediments (Table 1). The carbon isotopic signature of seagrass leaves from the same location has been recently reported as -7.96 ± 0.27 ‰ by Duarte et al. (2018).

3.2 Carbon dioxide and methane air-seawater fluxes

The daily CO_2 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 CO_2 uptake, although differences were not significant (Kruskal-Wallis, $p > 0.05$; Fig. 1A). At both sites, S1 and S2, the daily net CH_4 flux was 10- to 100-fold higher in vegetated compared to adjacent bare sediments with generally higher fluxes at S2 (Kruskal-Wallis, $p < 0.01$; Fig. 1B). The total CO_2 greenhouse-equivalent fluxes varied between sites and were higher in the vegetated compared to the bare sediments (Kruskal-Wallis, $p < 0.01$; Fig. 1C).

3.3 Effect of warming on carbon dioxide and methane air-seawater fluxes

The CO_2 fluxes in vegetated sediments increased greatly with warming ($R^2 = 0.38$, $p < 0.001$), but decreased over time when the community was maintained at 25 ˚C ($R^2 = 0.30$, $p < 0.01$; Fig. 2A, Table S1), shifting from sediments showing net CO_2 emission to net CO_2 uptake. Similar responses were observed in the bare sediments, where CO_2 fluxes increased with warming ($R^2 = 0.54$, $p < 0.0001$), while the community tended to shift over time from supporting net CO_2 emission to net CO_2 uptake when the maintained at 25 ˚C (Fig. 2B). The net CO_2 flux, i.e. the difference between the CO_2 fluxes in warming sediments and those at sediments maintained at 25 ˚C, increased significantly with warming in both the vegetated and the bare sediment ($R^2 = 0.74$, $p < 0.05$ and $R^2 = 0.91$, $p < 0.001$, respectively, Fig. 2C).

CH_4 fluxes declined over time when the sediments were maintained at 25 ˚C, both in vegetated ($R^2 = 0.43$, $p < 0.001$, Fig. 2D) and, less strongly, bare sediments ($R^2 = 0.24$, $p < 0.01$; Fig. 2E, Table S2). In contrast, CH_4 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 ($p > 0.05$ and $p > 0.05$, respectively; Table S2). The net CH_4 fluxes, i.e. the difference between the CH_4 fluxes in sediments exposed to warming and those sediments at maintained at 25 ˚C, increased significantly over time (i.e. with warming) in vegetated ($R^2 = 0.69$, $p < 0.05$) but not in bare sediments ($p > 0.05$; Fig. 2F). An outlier in the vegetated sediment at 33 ˚C supporting extreme emissions (CO_2 flux of 55,170 µmol CO_2 m^{-2} d^{-1} and CH_4 flux of 699.8 CH_4 µmol m^{-2} d^{-1}), was observed on day 14 in one of the replicates of the warming treatment where seagrass had died (Fig. 2A and D), and was excluded from the regression analyses reported above.

Despite CO_2 and CH_4 fluxes showing the same response to warming in both types of sediment, vegetated sediments held higher fluxes than bare sediments. The relationship between net CO_2 and CH_4 fluxes in bare vs. vegetated sediments showed that both bare and vegetated communities tended to act as net CO_2 sinks at 25 ˚C, but tended to act as CO_2 sources at warmer temperatures (Fig. 3A), whereas net CH_4 fluxes were 3- to 8-fold higher in vegetated compared to bare sediments. (Fig. 3B).

3.4 Effect of darkness on carbon dioxide and methane air-seawater fluxes

The vegetated sediment shifted over time from showing net CO_2 uptake to net CO_2 emission when maintained in the dark ($R^2 = 0.70$, $p < 0.05$), while the increase in the bare sediment was not significant ($p > 0.05$; Fig. 4A, Table S3). In contrast, when vegetated sediment was maintained at 25 ˚C at a 12 h L : 12 h D photoperiod, the community shifted from net CO_2 emission to net CO_2 uptake (Mann Whitney, $p < 0.05$; Fig. 5A). In bare sediments, CO_2 fluxes showed the same trend in cores maintained at 25 ˚C at a 12 h L : 12 h D photoperiod than under dark conditions (Fig. 5B).

When vegetated sediments were kept in the dark, net CH_4 fluxes decreased 5-fold over time ($R^2 = 0.99$, $p < 0.0001$; Fig. 4B, Table S4). However, the CH_4 fluxes did not differ significantly between vegetated cores maintained at 25 ˚C in the 12 h L : 12 h D photoperiod or in the dark (Mann Whitney, $p > 0.05$), showing the same trend of decreasing CH_4 fluxes (Fig. 5C). In the bare sediment, CH_4 fluxes in sediments kept in the dark were higher than those at 25 ˚C under a 12 h L : 12 h D photoperiod, with significant differences only observed on days 14 and day 20 (Mann Whitney, $p < 0.05$ and $p < 0.05$, respectively; Fig. 5D).

3.5 Isotopic composition of carbon dioxide ($\delta^{13}C$-CO_2) and methane ($\delta^{13}C$-CH_4)

The isotopic signature of the $\delta^{13}C$-CO_2 became heavier with warming in the bare sediment, increasing from -22.36 ± -4.97 ‰ $\delta^{13}C$ at 25 ˚C to -9.01 ± 0.98 ‰ $\delta^{13}C$ at 37 ˚C ($R^2 = 0.91$, $p < 0.001$), while the other treatments showed similar values over time, ranging from a minimum average of -11.55 ± 5.32 ‰ to a maximum average of -17.89 ± 1.81 ‰ $\delta^{13}C$ (Fig. 6A-D).

The isotopic signature of $\delta^{13}C$-CH_4 decreased over time in both vegetated and bare sediments, whether they were maintained at constant temperature or experienced warming (Fig. 6E-H). The isotopic signature in the vegetated sediment exposed to warming decreased significantly from -50.8 to -54.06 ‰ ($R^2 = 0.67$, $p < 0.001$).

The $\delta^{13}C$ isotopic composition of both CO_2 and CH_4 became heavier over time when the community was kept in the dark (Fig. 7), with a significant increase of $\delta^{13}C$-CH_4 in bare sediments ($R^2 = 0.94$, $p < 0.01$; Fig 7D).

4 Discussion

4.1 Sediment characteristics

The values reported for CO_2 and CH_4 fluxes varied greatly between the two sites studied here, with higher fluxes in the more organic sediments with higher biomass. Similar trends were seen by Garcias-Bonet and Duarte (2017) who reported an increase in CH_4 fluxes with increasing organic matter content in Red Sea seagrass sediments. Additionally, CO_2 and CH_4 fluxes were also highly variable over time in the studied site, as the first evaluation of CH_4 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 CO_2 and CH_4 fluxes between species and location (cf. Table 1 in Garcias-Bonet and Duarte (2017)). CO_2 fluxes were also 10-fold higher in vegetated compared to adjacent, but bare sediments, indicating elevated microbial remineralization rates in vegetated sediments.

4.2 Effect of warming

Vegetated sediments exposed to warming shifted from acting as a CO_2 sink to an increasingly intense source, while the CO_2 fluxes in vegetated sediments maintained at 25 ˚C decreased over time. Warming leads to an increase in both community photosynthesis and respiration, with respiration increasing at a faster rate (Harris et al., 2006) explaining the shift to a CO_2 source in sediments exposed to a thermal stressor. 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 CO_2 m^{-2} d^{-1}; Banerjee et al., 2018).

The results presented here add to those by Garcias-Bonet and Duarte (2017) to identify Red Sea seagrass communities as a significant source of CH_4. The presence of seagrass resulted in a higher organic matter supply to the sediments, favoring the presence of methanogens, which led to higher CH_4 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 CH_4 fluxes supported by vegetated compared to bare sediments in this study.

Mean CH_4 fluxes at *in situ* temperature (25 ˚C) in vegetated sediments were lower than the mean value of 85.09 ± 27.80 µmol CH_4 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 CH_4 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 CH_4 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 production rates at higher temperatures. We also reported a 10-fold decline in CH_4 fluxes over time for sediment communities maintained at 25 ˚C, which could be attributable to increased sulfate reduction, reduced CH_4 production or a combination of both. Methane is produced under anoxic conditions in marine sediments, yet only a small portion is released, as CH_4 production by methanogens is compensated for by CH_4 consumption by sulfate-reducing bacteria (Barnes and Goldberg, 1976).

The isotopic signature of the CO_2 released from bare sediments shifted with warming indicating a shift from seston (-25.43 ± 0.42 ‰; Duarte et al., 2018) as the organic matter supporting respiration to seagrass carbon (average $\delta^{13}C$ value of -7.73 ± 0.11 ‰ for Red Sea seagrass; Duarte et al., 2018) as the source of CO_2. 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. 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).

4.3 Effect of prolonged darkness

Communities maintained at 25 ˚C and a 12 h L : 12 h D photoperiod showed continuous net CO_2 uptake, while the communities kept in the dark shifted, as expected, to a heterotrophic state, acting as a CO_2 source. The net CO_2 production corresponded to community respiration rates, while that at 12 h L : 12 h D photoperiod corresponded to the net community production.

We found, however, no effect of prolonged darkness on CH_4 fluxes, suggesting that the elevated CH_4 fluxes in vegetated sediments were not directly supported by fresh photosynthetic products, but rather by the elevated organic matter content in vegetated sediments compared to bare ones. These findings were in contrast to those reported by Lyimo et al. (2018) who found increased CH_4 fluxes under shading.

4.4 Implications

Reports on greenhouse gas fluxes by seagrass ecosystems are limited (Oremland, 1975; Barber and Carlson, 1993; Alongi et al., 2008; Deborde et al., 2010; Bahlmann et al., 2015; Garcias-Bonet and Duarte, 2017; Banerjee et al., 2018; Lyimo et al., 2018), and no reports had been previously published on how increasing seawater temperatures might affect greenhouse gas fluxes by seagrass ecosystems. Blue carbon ecosystems have shown to turn into C sources when disturbances led to mortality (Macreadie et al., 2015; Lovelock et al., 2017; Arias-Ortiz et al., 2018), consistent with the very large CO_2 and CH_4 fluxes observed in one vegetated sediment where the seagrass died when warmed to 33 ˚C. However, even where seagrass remained alive, warming led to elevated greenhouse fluxes. In terms of CO_2 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. Additionally, the elevated nutrient and high organic matter stock in seagrass meadows, which supports a 1.7-fold higher organic matter content than surrounding bare sediments, can promote an increase in CO_2 and CH_4 fluxes following disturbance (Gonsalves et al., 2011; Sotomayor et al., 1994). Our results suggest that this stock in seagrass sediments may be remineralized to support net greenhouse gas fluxes at the warmer temperatures reached and with further warming of the Red Sea. Hence, warming may, as other disturbances (Lovelock et al., 2017), shift seagrass ecosystems from net sinks to net sources of greenhouse gases, thereby providing a feedback mechanism that may contribute to further enhance global warming.

5 Conclusion

In summary, this study reports, for the first time, experimental evidence that warming leads to increased greenhouse gas (CO_2 and CH_4) fluxes in a *H. stipulacea* meadow in the Red Sea, and may lead to seagrass meadows shifting from acting as sinks to sources of greenhouse gases. The elevated organic content and plant activity in vegetated sediments led to higher CO_2 and CH_4 fluxes in vegetated compared to bare sediments, and a much steeper increase in CO_2 and CH_4 fluxes with warming. In addition, prolonged darkness led to an increase in CO_2 fluxes, while CH_4 fluxes decreased over time, also indicating organic matter to be the driver. 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.

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Author contributions

NG-B and CMD designed the project. CB collected the samples and conducted the experiments. NG-B, CMD and CB analyzed the results, CMD and CB wrote the first draft of the manuscript, and all authors contributed substantially to the final manuscript. All authors approved the final submission.

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TABLES

Table 1. Summary of vegetated sediment, bare sediment and seagrass leaves characteristics in the study sites (S1 and S2). NA: Not available.

	S1			S2		
	Vegetated sediment	Bare sediment	Seagrass leaf	Vegetated sediment	Bare sediment	Seagrass leaf
C concentration (% DW)	0.43 ± 0.05	0.41 ± 0.03	17.6 ± 2.72	0.55 ± 0.03	0.52 ± 0.02	15.32 ± 1.48
N concentration (% DW)	0.07 ± 0.01	0.12 ± 0.01	1.06 ± 0.17	0.08 ± 0.002	0.09 ± 0.01	0.94 ± 0.07
P concentration (% DW)	0.03 ± 0.001	0.03 ± 0	0.12 ± 0.01	0.02 ± 0	0.02 ± 0.001	0.11 ± 0.01
Carbonate content (% DW)	91.75 ± 0.56	91.65 ± 0	NA	82.61 ± 0.50	83.63 ± 0	NA
Organic matter (% DW)	2.45 ± 0.09	2.46 ± 0.16	NA	3.26 ± 0.03	2.95 ± 0.04	NA
Bulk density (g cm^{-3})	1.15 ± 0.02	1.28 ± 0.03	NA	1.1 ± 0.07	1.2 ± 0.04	NA
Seagrass biomass (g DW m^{-2})	60.87 ± 1.24	NA	NA	164.66 ± 20.54	NA	NA
Sediment δ^{13}C–C$_{org}$ (‰)	-15.77 ± 0.07	-15.94 ± 0.1	NA	-15.81 ± 0.13	-16.36 ± 0.28	NA
Seagrass leaf δ^{13}C–C (‰), extracted from Duarte et al. (2018)	-7.96 ± 0.27					

FIGURES

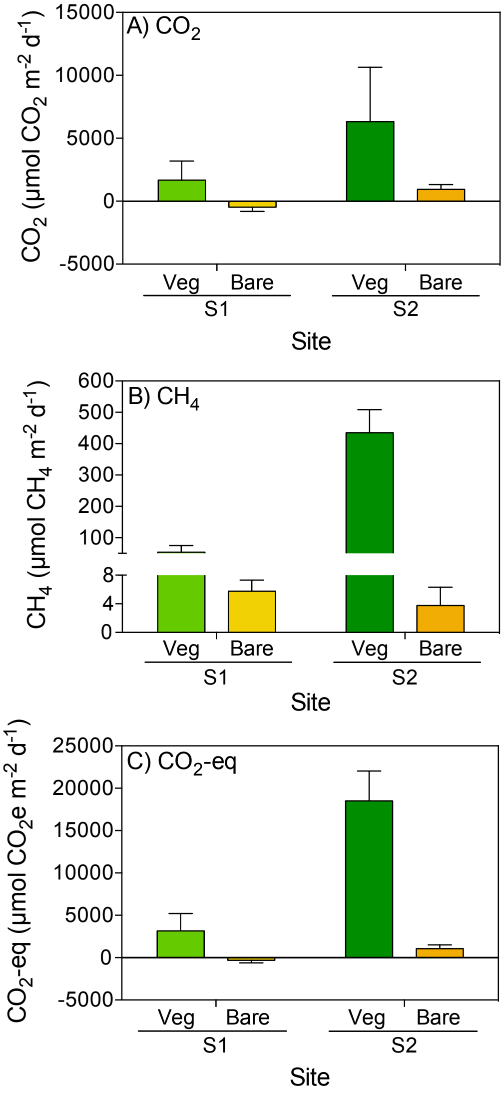

Figure 1: Mean + SE **(A)** CO_2, **(B)** CH_4, **(C)** $CO_{2\text{-eq}}$ fluxes in vegetated (green) and adjacent bare (yellow) sediments at two sites (S1 and S2) in the central Red Sea.

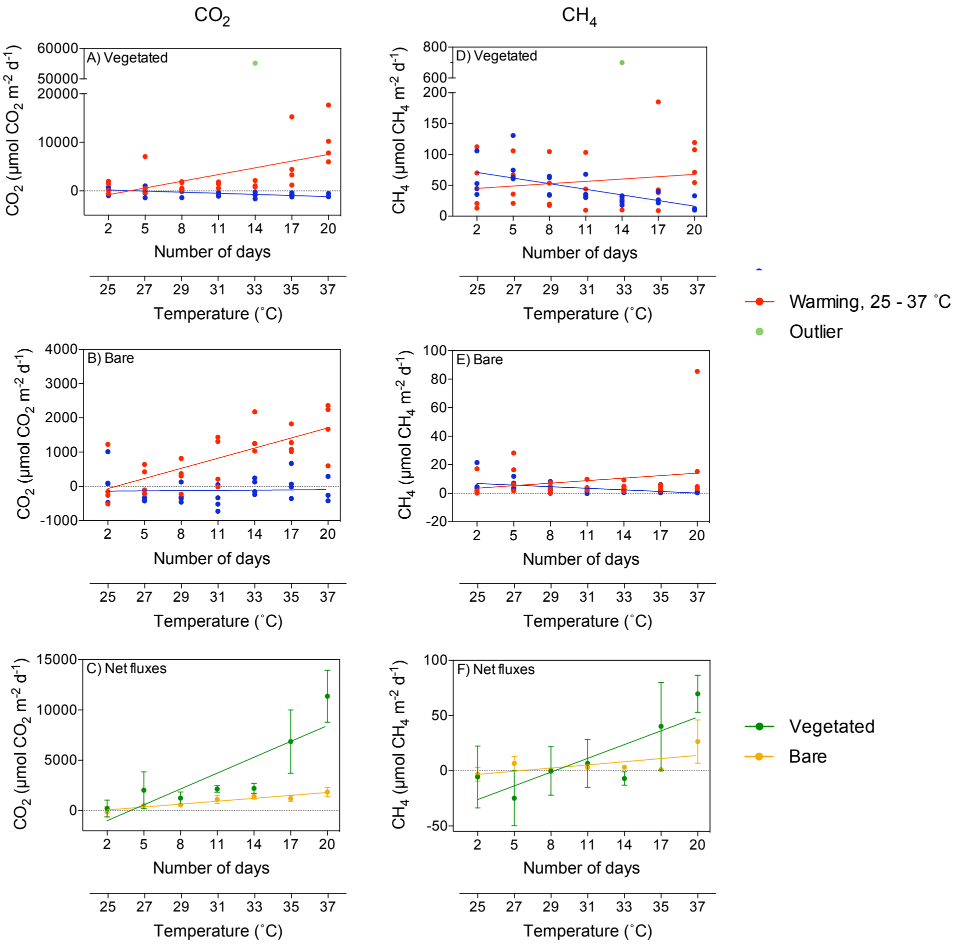

Figure 2: Mean ± SE CO$_2$ (left) and CH$_4$ (right) fluxes in **(A and D)** vegetated and **(B and E)** bare sediments. Symbols indicate each replicate of the community experiencing warming from 25 - 37 ˚C (red) and the community maintained at 25 ˚C (blue) over the experimental period (number of days since the onset of the experiment). An outlier at 33 ˚C in vegetated sediments in shown in green. **(C and F)** Mean ± SE CO$_2$ (C) and CH$_4$ (F) net fluxes in vegetated (green) and bare (yellow) sediments over the experimental period (number of days since the onset of the experiment). The second x-axis indicates the experimental temperature for the community exposed to warming from 25 - 37 ˚C.

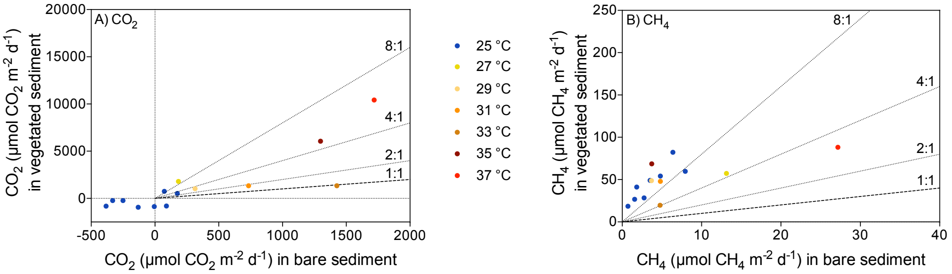

Figure 3: Relationship between vegetated and bare sediments for **(A)** CO$_2$ and **(B)** CH$_4$ fluxes. Symbols indicate different temperatures ranging from 25 - 37 °C, the dashed line indicates line 1:1, and dotted lines show lines 2:1, 4:1 and 8:1.

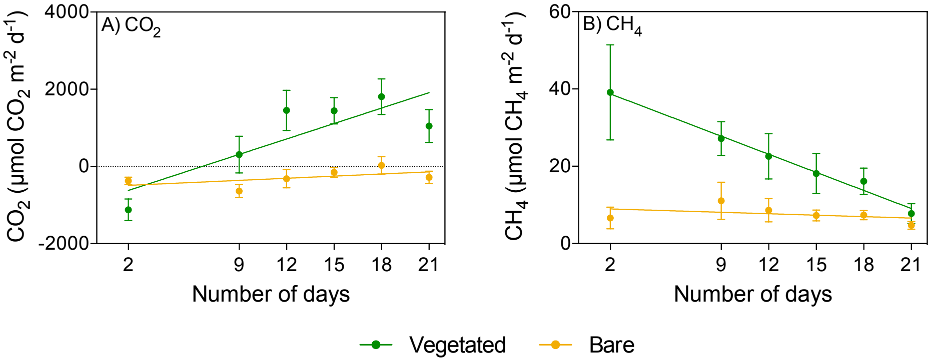

Figure 4: Mean ± SE **(A)** CO$_2$ and **(B)** CH$_4$ fluxes in vegetated (green) and bare (yellow) sediments of communities exposed to prolonged darkness over the experimental period (number of days since the onset of the experiment).

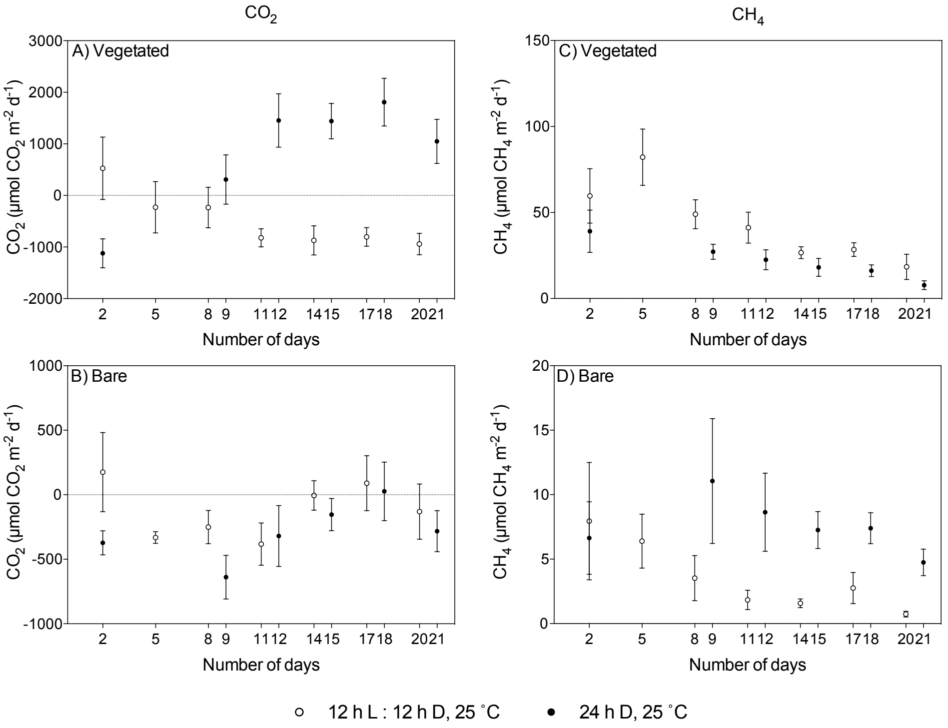

Figure 5: Comparison of mean ± SE CO$_2$ (left) and CH$_4$ (right) fluxes in **(A and C)** vegetated and **(B and D)** bare sediments maintained at 25 ˚C and a 12 h L:12 h D photoperiod (white) and communities kept at 25 ˚C and a 24 h D period (black) over the experimental period (number of days since the onset of the experiment). Dots indicate mean values and error bars indicate standard error of the mean.

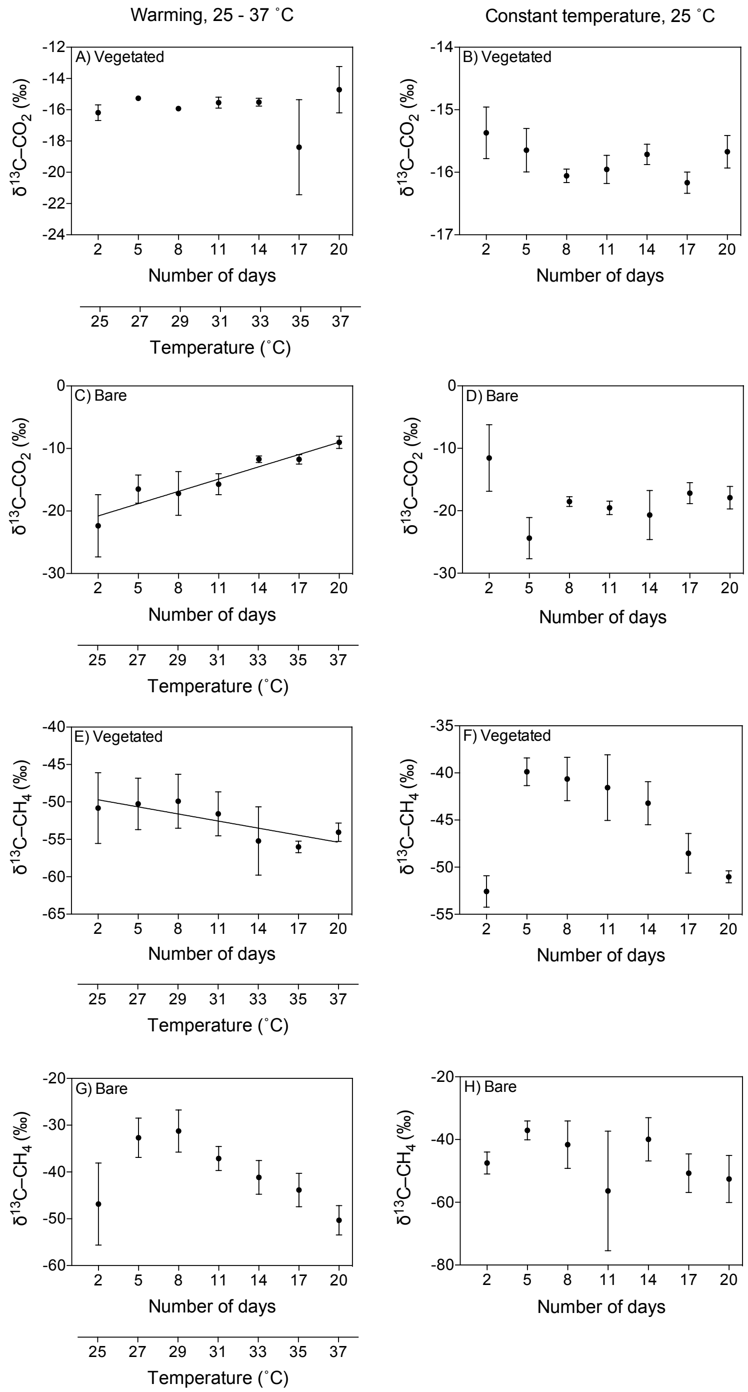

Figure 6: Mean ± SE isotopic signature of CO_2 ($\delta^{13}C$-CO_2) and CH_4 ($\delta^{13}C$-CH_4) in the communities experiencing warming from 25 - 37 ˚C (left) and the communities maintained at 25 ˚C (right). **(A-D)** $\delta^{13}C$-CO_2 is shown for the vegetated (A and B) and bare (C and D) sediments over the experimental period (number of days since the onset of the experiment). **(E-H)**

δ^{13}C-CH_4 is shown for the vegetated (E and F) and bare (G and H) sediment over the experimental period. The second x-axis indicates the temperature increase for the community experiencing warming.

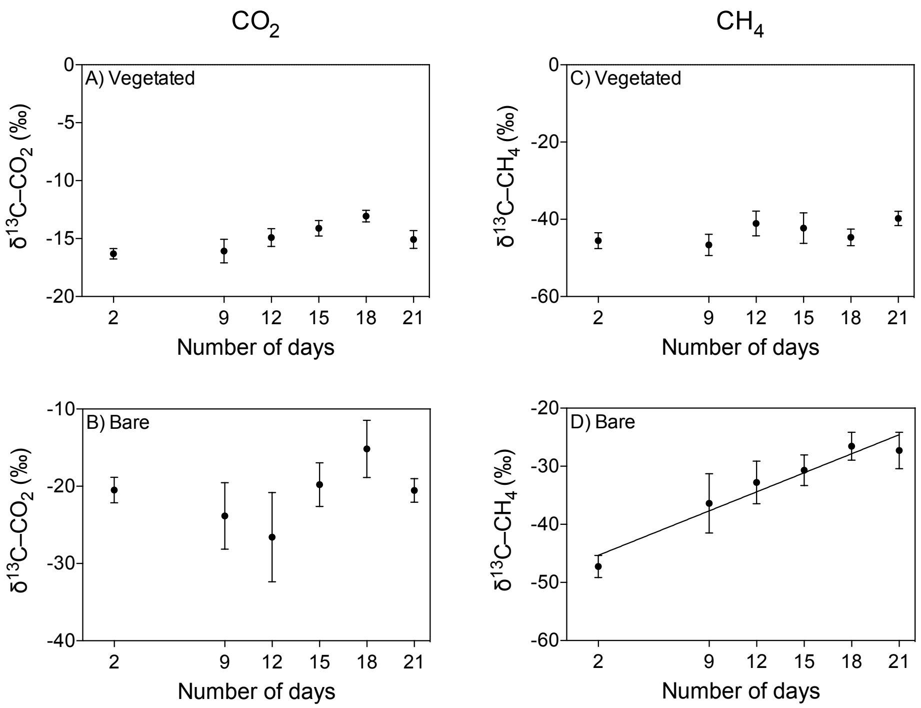

Figure 7: Mean ± SE isotopic signature of CO_2 (δ^{13}C-CO_2, left) and CH_4 (δ^{13}C-CH_4, right) in **(A and C)** vegetated and **(B and D)** bare sediments exposed to prolonged darkness over the experimental period (number of days since the onset of the experiment).