Soil Biology and Biochemistry 157 (2021) 108235

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: http://www.elsevier.com/locate/soilbio

Interactive effects of temperature and redox on soil carbon and iron cycling

Nikhil R. Chari [a,*,1], Yang Lin [a,b], Yuan S. Lin [a], Whendee L. Silver [a]

[a] Department of Environmental Science, Policy, and Management, UC Berkeley, 130 Mulford Hall #3114, Berkeley, CA, USA, 94720
[b] Department of Soil and Water Sciences, University of Florida, 2181 McCarty Hall, Gainesville, FL, USA, 32611

ARTICLE INFO

Keywords:
Methane
Iron reduction
Redox
Temperature
Microbial biomass
Carbon
Puerto Rico
Sacramento-San Joaquin Delta
Tropical forest
Temperate peatland

ABSTRACT

Shifts in temperature and rainfall regimes with climate change can feed back on redox dynamics and associated biogeochemical cycling in humid environments. This has global implications for greenhouse gas emissions and ecosystem carbon (C) storage and loss. Though aerobic carbon dioxide (CO_2) production is generally understood to follow an Arrhenius-type temperature dependence, the temperature sensitivity of anaerobic metabolisms such as iron (Fe) reduction and methane (CH_4) fluxes, both processes affecting C cycling in soils, remains unclear in upland soils. We used a fully factorial incubation experiment to examine the interactive effects of temperature (8, 26, and 35 °C) and redox (oxic and anoxic) conditions on CO_2 production, CH_4 fluxes, Fe reduction, microbial biomass C, and the microbial metabolic quotient (MMQ) in upland soils from two contrasting climate regimes and redox environments: a tropical forest in Puerto Rico (PR) and a temperate peatland in California (CA). Under oxic conditions, we found that CO_2 production increased across both temperature gradients (8–26 °C and 26–35 °C). In contrast, under anoxic conditions, net CH_4 fluxes and Fe reduction exhibited a greater temperature sensitivity between 26 and 35 °C than 8–26 °C in both PR and CA soils. We also observed coupling between Fe reduction and net CH_4 fluxes in PR and CA soils consistent with competition for acetate. The anoxic MMQ increased in PR soils at high temperature, but not in CA soils, suggesting that the native PR environment may favor the development of more efficient microbial communities better able to tolerate warmer, wetter conditions. Together, our results suggest that anaerobic microbial metabolisms may be much more sensitive to higher temperature than aerobic ones, possibly due to proportionally greater anaerobic microbial activity at high temperature. Our results suggest that increased global temperatures combined with higher rainfall regimes may significantly increase anaerobic greenhouse gas production in humid environments.

1. Introduction

Climate models project increases in extreme rainfall events in some humid terrestrial environments as a result of rising atmospheric greenhouse gas (GHG) emissions (Chadwick et al., 2016; Donat et al., 2016). Many of these environments, such as tropical forests and peatlands, play a significant role in global carbon (C) exchange and can function as C sources or sinks, depending on climatic conditions (Kayranli et al., 2010; Taylor et al., 2017). Higher precipitation with climate change can increase the occurrence of anoxic or low redox environments (McNicol and Silver 2014, Liptzin and Silver, 2009); in these regions, anaerobic soil metabolisms are likely to become more common. Aerobic production of carbon dioxide (CO_2) in soil has been shown to increase exponentially with rising temperature as predicted by the Arrhenius equation (Lloyd and Taylor, 1994; Davidson et al., 2012), or exhibit an otherwise positive correlation (Fang and Moncrieff, 2001; Sierra, 2012). However, less is known about temperature effects on anaerobic processes impacting the C cycle such as iron (Fe) reduction and methanogenesis, particularly in upland soils of temperate and tropical ecosystems. Understanding the simultaneous temperature and redox sensitivity of these processes is particularly important for predicting their response to climate change.

Iron reduction can be an important driver of CO_2 emissions and soil C storage (Baldock and Skjemstad, 2000; Liptzin and Silver, 2009; Hall and Silver, 2015). The production of Fe(II) can result in the solubilization of minerally-bound soil organic C, increasing the likelihood of respiration (Berhe et al., 2012; Buettner et al., 2014). Previous research suggested that short term rates of soil CO_2 production were similar under oxic and anoxic conditions in Fe-rich humid tropical forest soils (DeAngelis et al., 2010; Gross et al., 2018). This is surprising as

* Corresponding author.
E-mail addresses: nchari@g.harvard.edu (N.R. Chari), ylin2@ufl.edu (Y. Lin), ysl.yuanlin@berkeley.edu (Y.S. Lin), wsilver@berkeley.edu (W.L. Silver).
[1] Current address: Department of Organismic and Evolutionary Biology, Harvard University, 1300 Centre St, Boston, MA, USA, 02131.

https://doi.org/10.1016/j.soilbio.2021.108235
Received 14 November 2020; Received in revised form 12 February 2021; Accepted 23 March 2021
Available online 30 March 2021
0038-0717/

microbial respiration has long been considered to decrease significantly under anoxic conditions (Greenwood, 1961). The relatively high rate of soil respiration in tropical forest soils may be driven in part by dissimilatory microbial Fe reduction coupled to organic C oxidation (Canfield et al., 1993; Stemmler and Berthelin, 2003). These soils are typically characterized by fluctuating redox due to warm, perhumid conditions, finely textured and organic-rich soils, and oxygen consumption that exceeds diffusive resupply (Silver et al., 1999), and rates of Fe reduction can be accelerated by these redox fluctuations in the tropics (Ginn et al., 2017). A similar effect has been described in Fe-rich drained temperate peatlands that also feature fluctuating redox conditions (Yang and Liptzin, 2015; Lovley and Phillips, 1986).

Given the importance of Fe reduction in soil C dynamics, its coupled temperature and redox sensitivity merits further research (Coward et al., 2018). Under controlled laboratory conditions, Fe reduction increased exponentially with temperature in highly acidic mine pit lake sediments (Meier et al., 2005), and dissimilatory microbial Fe(III) reduction increased at higher temperatures in a sub-alpine wetlands (Schilling et al., 2019). However, the effects of temperature on anaerobic C fluxes are poorly understood in Fe-rich tropical forests or temperate peatlands, both of which hold considerable C stocks.

Methane (CH_4) is a potent GHG with approximately 34–86 times the global warming potential of CO_2 (IPCC, 2014). Previous studies of the response of CH_4 fluxes to warming have largely focused on subarctic peatlands and tundra (White et al., 2008; Turetsky et al., 2008; Voigt et al., 2017). These studies observed small increases in CH_4 production with temperature over a range of 0.5–4 °C of warming. However, higher Q_{10} values and more variability have been observed when Arctic and sub-arctic peatland, boreal, and tundra soils were warmed across a wider range of temperatures, with Q_{10} values spanning from 1.3 to 6 with outliers upwards of 20 (Lupascu et al., 2012; Treat et al., 2015; Zheng et al., 2018). An Alaskan tundra soil incubated over a 10 °C temperature range exhibited Q_{10} values for methanogenesis consistently above 5, and as high as 22 (Roy Chowdhury et al., 2015). The upper limits of these values were higher than the Q_{10} values typically associated with CO_2 production (Wang et al., 2010), suggesting that methanogenesis could have a greater temperature sensitivity than CO_2 production, while the median Q_{10} values in boreal and tundra soils were lower for net CH_4 fluxes (median $Q_{10} = 1.16$) than for CO_2 production (median $Q_{10} = 1.39$) (Treat et al., 2015). In studies of methanogenic bacterial communities temperature increases led to overall decreases in the abundance of methanogens, while having no effect on community composition (Peltoniemi et al., 2016; Cui et al., 2015). How soil CH_4 fluxes respond to temperature along broader temperature gradients in tropical or temperate environments is not well understood.

Methanogens and Fe-reducing microbes can both use acetate as a substrate, and methanogenic access to soil acetate can be suppressed by Fe reduction (Teh et al., 2008). In Arctic tundra soils, acetate consumed in methanogenesis increased with temperature, whereas acetate consumed in Fe reduction was temperature-independent (Herndon et al., 2015). It is unclear if this is a widespread phenomenon and understanding the temperature dynamics of Fe reduction and their relationship with CH_4 fluxes is relevant to predicting soil C storage and GHG fluxes under a changing climate.

Here, we used a fully factorial incubation experiment to explore the separate and combined effects of temperature and redox conditions on key aspects of C cycling including soil CO_2 production, CH_4 fluxes, Fe reduction, and microbial biomass C, as well as microbial metabolic quotient. We tested the hypothesis that anaerobic soil metabolisms would exhibit different sensitivity to temperature changes than CO_2 production due to differential temperature responses of Fe reduction and methanogenesis, and their potential interactions. We used Fe-rich soils from a tropical forest and temperate peatland to explore the potential effects of native climo-edaphic conditions on the processes of interest.

2. Materials and methods

2.1. Study sites

Samples (10 kg each, 0–10 cm depth) were collected from two sites with Fe-rich soils and fluctuating redox conditions, but different bioclimatic conditions: a humid tropical forest in Puerto Rico (PR) and a flood-irrigated peatland soil in California (CA) (Table 1). Samples were collected over a small area (approximately 5×5 m^2) in each location and pooled within sites (CA or PR). Separate experiments were carried out on PR and CA soils to accommodate space in the glovebox. Tropical soil samples were taken from the El Verde research area (18.3° N, 65.8° W) in the Luquillo Experimental Forest, PR at approximately 380 m a.s.l. The tropical soil was an Ultisol (Beinroth, 1982) and vegetation was characterized as the tabonuco forest type with a mean annual temperature of approximately 23 °C, and a mean annual precipitation of approximately 3200 mm (Weaver and Murphy, 1990; Murphy et al., 2017). The site is part of the NSF-sponsored Long-term Ecological Research program and a Critical Zone Observatory and Thematic Cluster. The peatland site was an alfalfa cropland on Mollisols in the Sacramento/San Joaquin River Delta, CA (38.1 °N, 121.5 °W) at about −4 m a.s.l. with a mean annual temperature of 15 °C and a mean annual precipitation of approximately 330 mm (Hatala et al., 2012). The Mollisol developed on top of a deep buried peat layer. The site is believed to have been last drained in the late 1800s, and has been cultivated with alfalfa since 2015, and previously with corn. The soil bears resemblance to the Peltier series, characterized by deep, poorly drained soil formed in alluvium and aquatic plant remains.

Soil from the CA site contained higher levels of total C, HCl-extractable Fe, and clay content than soil from the PR site (Anthony and Silver, 2020; Gutiérrez del Arroyo and Silver, 2018; O'Connell et al., 2018) (Table 1). The peatland soil was periodically irrigated, but had a lower water holding capacity than the tropical forest soil (0.86 ± 0.02 g water g^{-1} dry soil for CA vs. 1.07 ± 0.01 g g^{-1} for PR, $p < 0.05$).

2.2. Incubation setup

Soils were homogenized gently by hand and large roots and rocks were removed. Approximately 100 g of soil was weighed into 473 mL mason jars. Moisture was adjusted to 60% water holding capacity at the start of the experiment (0.65 g water g^{-1} for PR; 0.52 g water g^{-1} for CA). Jars were kept covered in between gas sampling events (which occurred every 3 days) to minimize water loss. The incubation experiment followed a fully factorial design including two sites (PR and CA), two redox treatments (oxic and anoxic), and three temperature treatments (8 °C, 26 °C, and 35 °C) for a total of 12 treatments and 144 mesocosms; for each of the 12 treatments, $n = 6$ samples for gas sampling and an additional $n = 6$ samples for destructive sampling. Soils were incubated in a cold room (8 °C), at room temperature (26 °C average, over a range of 23–27 °C), and in a 35 °C incubator. Anoxic

Table 1
Site characteristics for PR and CA sites. Information is from Gutierrez del Arroyo and Silver (2018) and O'Connell et al. (2018) for PR and Anthony and Silver (2020) for CA, except for C, which was measured in this experiment.

Name	Location	pH	Total C (%)	Total N (%)	HCl-extractable Fe (mg/g)	Clay (%)
PR	El Verde research area, Luquillo Experimental Forest, Puerto Rico	4.89 ± 0.07	3.17 ± 0.01	0.40 ± 0.05	1.70 ± 0.48	22 ± 3
CA	Sacramento-San Joaquin Delta, California	4.93 ± 0.04	4.19 ± 0.01	1.00 ± 0.02	9.58 ± 1.43	27 ± 3

environments were prepared by evacuating the jar headspace and replacing it with an N_2:H_2 (95:5%) gas mixture using an airlock attached to an anaerobic glove box. Di-hydrogen can be a substrate for methanogenesis, but flux rates measured here were similar to those for soils from the same sites incubated in a pure N_2 headspace at ambient temperatures (Teh et al., 2008; McNicol and Silver 2014). Jars were fitted with gas-tight lids with gas sampling ports with rubber septa. Four-week long incubations for PR and CA soils were run sequentially for a total experimental period of 8 weeks.

2.3. Gas sampling

Headspace gas samples (30 mL) were taken once every three days over the course of four weeks and stored in pre-evacuated 20 mL glass vials for gas chromatography (GC) analysis. Vials were slightly overpressurized and sealed with silicone to minimize any chances of contamination from atmospheric air. For each oxic mesocosm, the mesocosm headspace was vented directly to the atmosphere for 10 min, and then resealed. An initial (T_0) sample was taken directly after resealing to determine baseline gas levels; the jar was then briefly opened and sealed again to equilibrate pressure and then left sealed for 3 h at which time a final (T_F) sample was collected. For anoxic samples, we took six T_0 samples from the glove box atmosphere during each gas sampling event, and T_F samples were taken from each re-sealed mesocosm headspace 3 h after venting. Anoxic samples were vented by evacuating the jar headspace in the airlock of the glove box with three evacuation cycles. Each cycle included pulling a vacuum of 0.67 atm and purging back to 0.03 atm with N_2 (first two cycles) and the glove box air (last cycle). After venting, anoxic mesocosms were transferred from the anaerobic airlock to the glovebox and allowed to equilibrate with the glove box atmosphere before resealing. Gas samples were analyzed on an Agilent 7890B GC (Agilent, Santa Clara, CA) for CO_2 and CH_4 using TCD and FID detectors, respectively, within 1 week of sampling or sealed with silicone to prevent leakage. Gas concentrations (ppm) were converted to gas production rates (μmol CO_2 g^{-1} soil h^{-1}) using the following equation:

$$R = 1000(c \cdot mol(T))/(m \cdot t) \quad (1)$$

Where *R* is the gas production rate (μmol [CO_2 or CH_4] g^{-1} soil h^{-1}), *c* is the gas concentration (ppm or mol [CO_2 or CH_4] per 10^6 mol gas), *mol(T)* is the number of moles of gas as a function of treatment temperature solved using the ideal gas law, *m* is the mass of dry soil, and *t* is the time of gas accumulation.

2.4. Soil analyses

Destructive samples for HCl-extractable Fe(II) and total HCl-extractable Fe were repeatedly collected from the same set of jars at the end of each week throughout the 4 week period. Iron was extracted from soil samples using 0.5 M HCl at 1:10 soil:HCl ratio by vortexing 4 g oven dry weight equivalent soil with 40 mL HCl and shaking overnight. Iron(II) in HCl was measured using the Ferrozine method and total HCl Fe was measured by reducing Fe(III) with hydroxylamine hydrochloride and measuring Fe(II) by the Ferrozine method (Viollier et al., 2000). Microbial biomass C was measured using the chloroform fumigation method (Brookes et al., 1985). Approximately 20 g of soils from each replicate at the end of the first and final weeks of the experiment were split into a 10 g control and a 10 g fumigation sample. The fumigation samples were held under vacuum with chloroform in a desiccator for at least 5 days. Dissolved organic C was extracted from control and fumigation samples with 40 mL 0.5 M K_2SO_4 in centrifuge tubes by shaking for approximately 1 h. The liquid phase was filtered (Whatman #1, pore size 11 μm) and stored in the freezer for dissolved organic C analysis on a TIC/TOC analyzer (O–I Analytical, College Station, TX). Microbial biomass C (MBC) was measured by subtracting control total organic C from fumigated total organic C. Microbial biomass C values were divided by 0.45 based on Brookes et al. (1985). The microbial metabolic quotient (MMQ) was calculated by standardizing average CO_2 fluxes on a given day per unit of average microbial biomass measured from soil destructively sampled the same day, resulting in a single value per treatment (Xu et al., 2017). Soil C concentrations were measured in duplicate on air-dried and ground soil on an elemental analyzer (CE Elantech, Lakewood, NJ). All analyses were performed at U.C. Berkeley.

2.5. Statistical analyses

Statistical analyses were carried out in R using additional packages "car" and "agricolae". Gas flux Q_{10} calculations were performed using a linear model plotting the natural log of flux rate against temperature based on the equation:

$$R = Ae^{bT} \quad (2)$$

Where *R* is the gas flux rate, *A* is the Arrhenius constant, *T* is the temperature, and *b* is the slope of the linear model such that $e^{(10b)}$ is the Q_{10} value (Zhou et al., 2013). ANOVA was used to determine the effects of temperature and headspace conditions on cumulative CO_2 production and CH_4 fluxes, Fe(II), and microbial biomass. Data met the ANOVA model assumptions, except for CH_4 and Fe(II) data, which exhibited non-normally distributed residuals due to higher variance in the anoxic 35 °C treatment. To normalize the residuals, an inverse square (y^{-2}) transformation was used on the dependent variables and ANOVA was conducted on the transformed data. A Tukey HSD test was used to determine the degree of significance between specific temperature and redox treatment interactions. Statistical significance was determined as the 90% level ($p < 0.1$) to account for large effect sizes despite sample heterogeneity, unless otherwise noted.

3. Results

3.1. Carbon dioxide fluxes

Cumulative CO_2 production followed an expected positive response to temperature in all treatments. Here, we defined the cumulative CO_2 production as the summed net CO_2 fluxes measured during 3-h periods every 3 days over the course of the 4-week experiment. In both PR and CA soils, cumulative CO_2 production increased significantly between both 8 and 26 °C and 26 and 35 °C ($p < 0.1$) (Fig. 1). In PR soils, oxic and anoxic CO_2 production exhibited similar temperature sensitivity, with Q_{10} values of 1.25 ± 0.03 and 1.28 ± 0.06, respectively (Fig. 1a). The temperature sensitivity of oxic and anoxic CO_2 production was also similar in CA soils, with Q_{10} values of 1.29 ± 0.02 and 1.36 ± 0.09, respectively (Fig. 1b). Total oxic CO_2 production was significantly higher than under anoxic conditions ($p < 0.05$) in PR soils, but there was no significant difference by redox treatments in CA soils.

3.2. Methane fluxes and Fe reduction

Headspace CH_4 and soil Fe(II) concentrations measured from oxic mesocosms were uniformly low across temperature treatments (Fig. 2). In the anoxic PR treatment, both cumulative CH_4 fluxes (calculated as the sum of all net CH_4 fluxes measured over the course of the experiment) and total reduced Fe(II) (measured at the end of the experiment) significantly increased over 13-fold between 26 and 35 °C ($p < 0.05$) but showed no significant change between 8 and 26 °C (Fig. 2a and b). Anoxic CH_4 and Fe(II) concentrations in CA soils did not exhibit a statistically significant increase between 26 and 35 °C; however, the difference between oxic and anoxic samples was greatest at 35 °C ($p < 0.001$) (Fig. 2c and d). Mean CH_4 fluxes in CA soils increased by over 400% between 26 and 35 °C as opposed to 30% between 8 and 26 °C (Fig. 2c). Methane fluxes and Fe(II) concentrations were positively

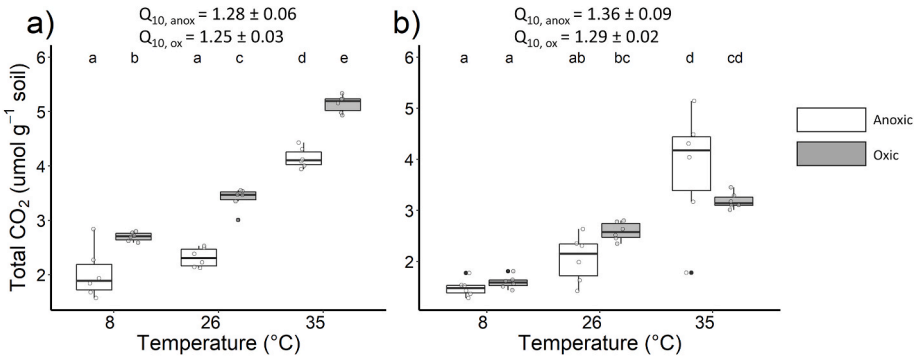

Fig. 1. Cumulative CO_2 production. The sum of fluxes measured at each sampling period over the 4-week experiment for Puerto Rico (a) and California (b) soils in anoxic and oxic environments. Different lower case letters denote statistically significant differences across treatments ($p < 0.1$). The box plots indicate the median and the first and third quantiles. The whiskers indicate the smallest and largest observations, discounting outliers which are marked by a black dot.

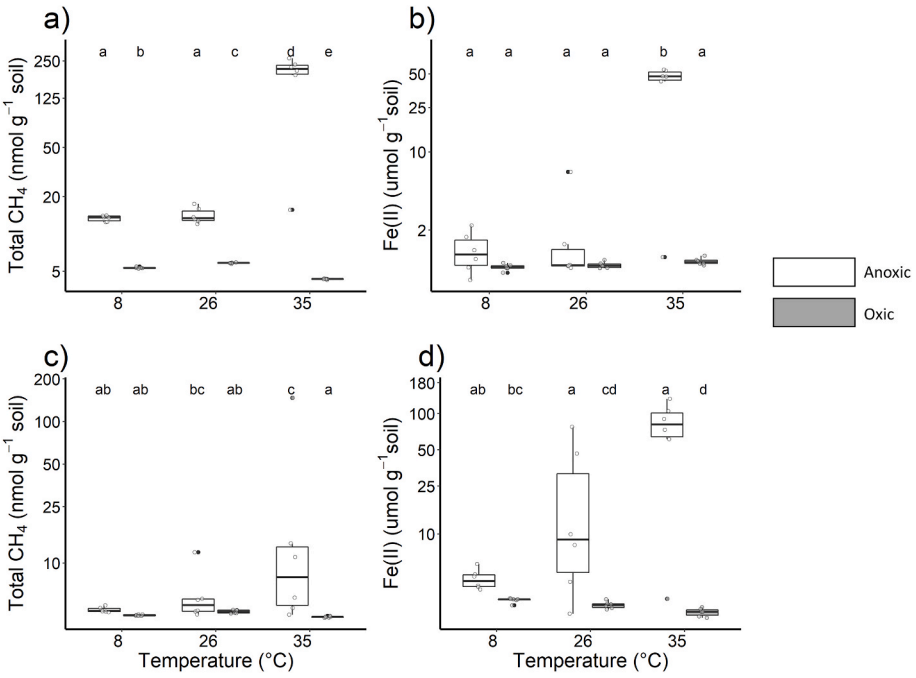

Fig. 2. Cumulative methane flux and reduced iron. Net methane (CH_4) flux (sum of measured fluxes from each sampling period over the experiment) (a, c) and total reduced iron (Fe(II)) concentrations at the end of the experiment (b, d) in incubations for PR (a, b) and CA (c, d) soils. Y-axes are log scale. Different lower case letters denote statistically significant differences across treatments ($p < 0.1$). The box plots indicate the median and the first and third quantiles. The whiskers indicate the smallest and largest observations, discounting outliers which are marked by a black dot.

correlated in both soils ($p < 0.05$, $R^2 = 0.98$ for PR, $R^2 = 0.36$ for CA). In PR soils, both CH_4 fluxes and Fe(II) concentrations increased throughout the experiment, whereas in CA soils, Fe(II) appeared to plateau after the second week of measurement, but methane fluxes continued to increase but with high variability (Fig. 3). In both PR and CA soils, CH_4 fluxes at 35 °C were more variable than at any other temperature.

3.3. Microbial biomass carbon and microbial metabolic quotient

Soil C concentrations were 3.17% ± 0.01% in the tropical soil and 4.19% ± 0.01% in the peatland soil. Microbial biomass C (MBC) concentrations were determined after the first and fourth weeks of incubation. No statistically significant differences were found in PR soil MBC concentrations across treatments after 1 week (Fig. 4a). After 4 weeks, however, MBC concentrations decreased significantly from 26 to 35 °C and from oxic to anoxic treatments ($p < 0.05$) (Fig. 4b). In CA soil, the anoxic 35 °C incubations exhibited significantly higher MBC than at lower temperatures after one week ($p < 0.001$) (Fig. 4c). In contrast with PR soil, MBC concentrations did not decrease between 26 and 35 °C after four weeks (Fig. 4d).

Average soil CO_2 production measured at the end of week 1 and week 4 were standardized by average microbial biomass to create a single microbial metabolic quotient measurement (MMQ) for each treatment. All calculated MMQ values for PR exhibited an increase in temperature sensitivity measured as higher Q_{10} values compared to total CO_2 production (Fig. 5). The increase in MMQ between 26 and 35 °C was more than 1.5 times greater in anoxic than oxic incubations. However, in CA soil, Q_{10} values decreased when standardized for MMQ in three out of four treatments (Fig. 6). Notably, CA MMQ did not increase with temperature in anoxic incubations, and exhibited a Q_{10} value less than 1 after 4 weeks, suggesting a decrease in MMQ with temperature (Fig. 6d). In many cases, uncertainty in Q_{10} increased after standardizing for MMQ.

4. Discussion

4.1. Temperature sensitivity of anaerobic metabolisms

Methane fluxes and Fe reduction, important anaerobic biogeochemical processes measured in this experiment, exhibited different sensitivities to temperature changes than aerobic CO_2 production in both a tropical forest and a temperate peatland, suggesting that

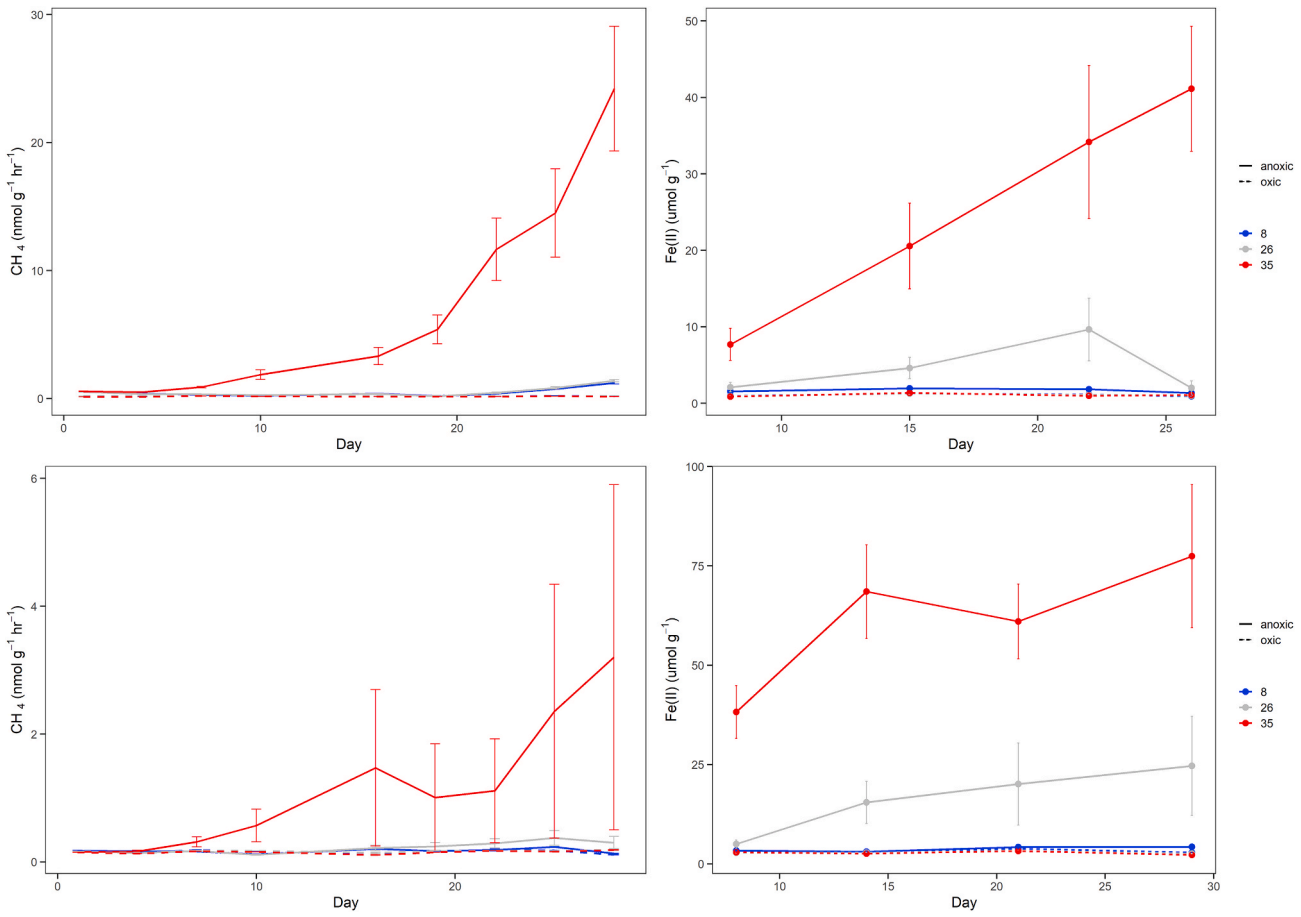

Fig. 3. Methane flux and Fe(II) concentrations over time. Net CH_4 fluxes (left) and Fe(II) concentrations (right) for PR (a, b) and CA (c, d) soils. Values are means and standard errors. CH_4 and Fe(II) were correlated in both PR ($p < 0.001$, $R^2 = 0.98$) and CA ($p < 0.001$, $R^2 = 0.36$) soils.

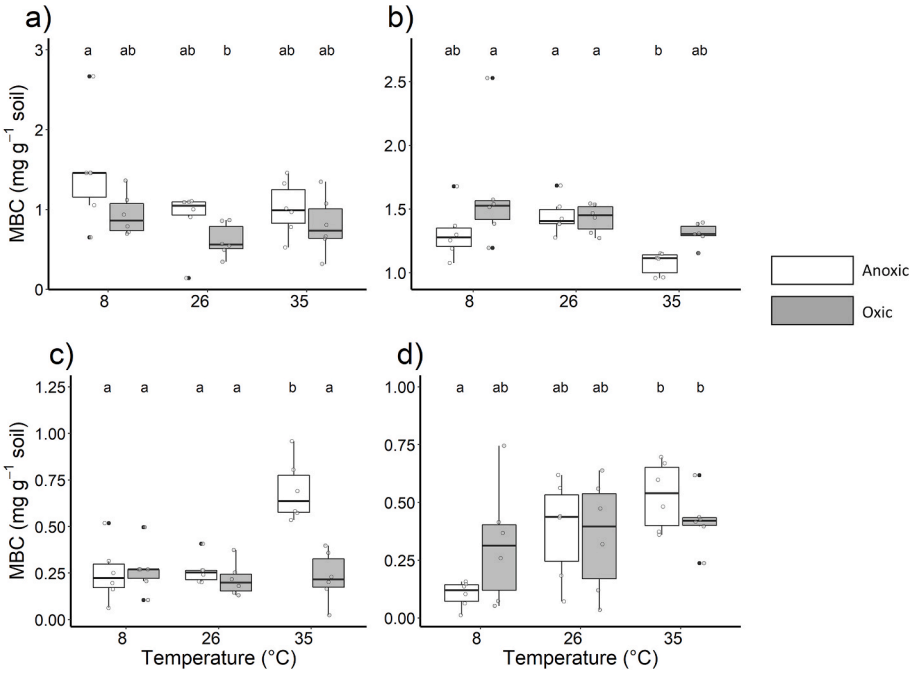

Fig. 4. Microbial biomass C (MBC). MBC measured after the first (a, c) and fourth (b, d) week of PR (a, b) and CA (c, d) soil incubations. Different lower case letters denote statistically significant differences across treatments ($p < 0.1$). The box plots indicate the median and the first and third quantiles. The whiskers indicate the smallest and largest observations, discounting outliers which are marked by a black dot.

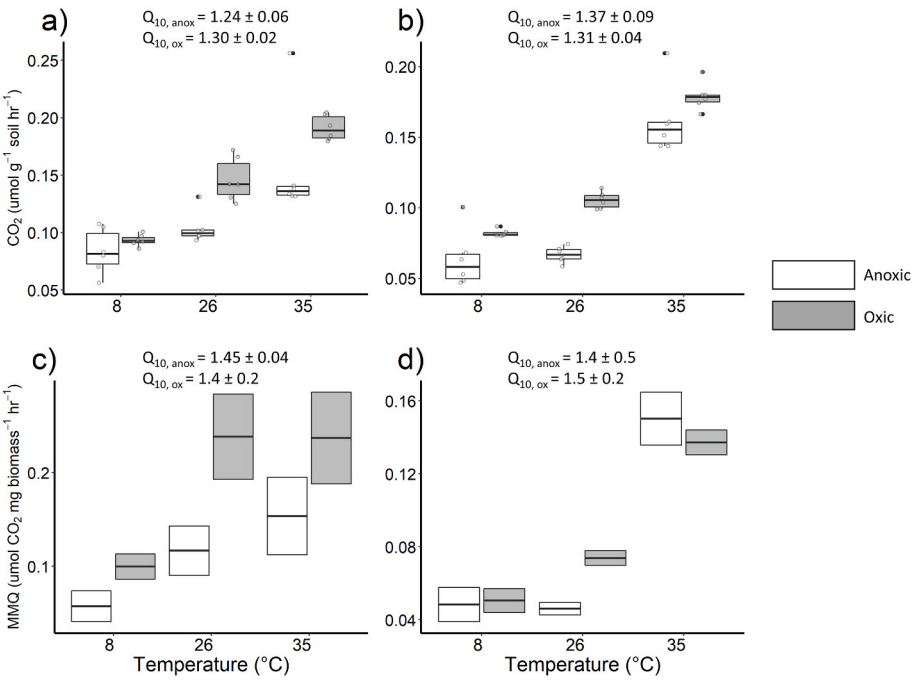

Fig. 5. Microbial metabolic quotient (MMQ) for Puerto Rico soils. CO_2 production after the first (a, c) and fourth (b, d) week of Puerto Rico (PR) soil incubation before (a, b) and after (c, d) being standardized by MBC measurements from the same day to produce an MMQ. Box plots of MMQ show the mean CO_2 production divided by the mean MBC measured on the same day as a single value. Lower and upper limits are calculated as the sum of the percentage standard errors of CO_2 respiration and MBC.

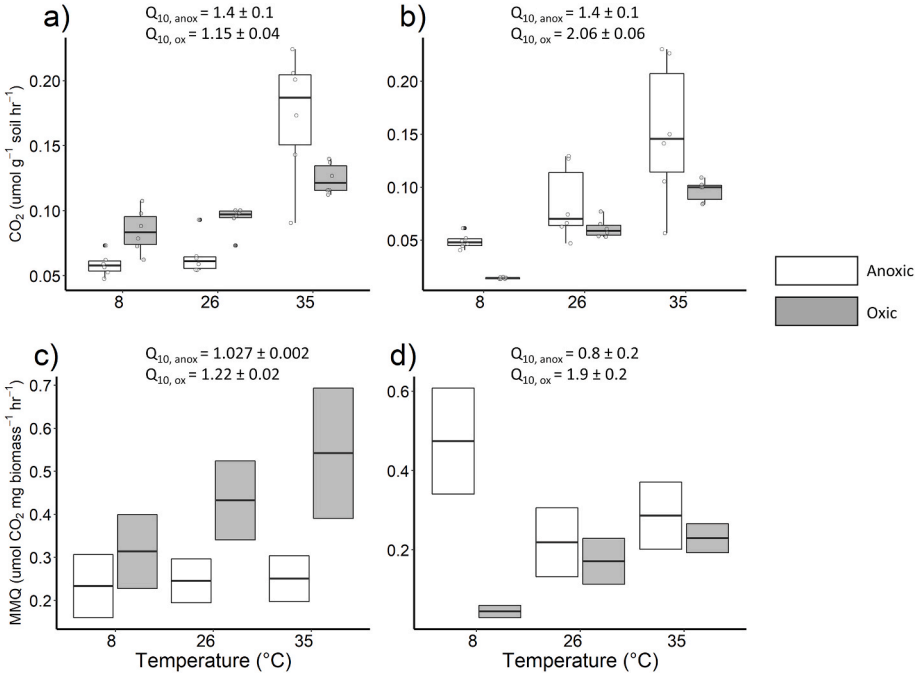

Fig. 6. Microbial metabolic quotient (MMQ) for California soils. MMQ values calculated for California (CA) soils (c, d) compared with original CO_2 production (a, b) after first (a, c) and fourth (b, d) weeks of incubation. Box plots of MMQ show the mean CO_2 production divided by the mean MBC measured on the same day as a single value. Lower and upper limits are calculated as the sum of the percentage standard errors of CO_2 production and MBC.

anaerobic metabolisms exhibit a significantly increased temperature sensitivity at higher temperatures. Neither CH_4 fluxes nor Fe reduction exhibited a significant increase with temperature between 8 and 26 °C in anoxic PR or CA soils. We found significant increases between 26 and 35 °C for both Fe(II) concentrations and CH_4 fluxes in PR soil, and the increase between 26 and 35 °C was consistently greater than between 8 and 26 °C for both processes at each site. In CA soil, the increase between oxic and anoxic CH_4 and Fe(II) was most significant at 35 °C. These results differ from past measurements that focused on aerobic CO_2 production and exhibited an exponentially or otherwise consistently increasing response to temperature (Lloyd and Taylor, 1994; Fang and Moncrieff, 2001). Our results suggest that anaerobic processes may exhibit a greater temperature sensitivity at higher temperatures than lower temperatures.

In both PR and CA soils, we observed anoxic CO_2 production at the same order of magnitude as under oxic conditions. This is similar to recent findings in tropical forests (Gross et al., 2018), although anoxic CO_2 production was much closer to oxic rates in CA soil than in past incubation measurements of anoxic peatlands (Scanlon and Moore, 2000). There were no site or treatment effects on the Q_{10} of cumulative

CO_2 production. Interestingly, we did not observe the same significant increase in CO_2 production between 8 and 26 °C under anoxic conditions in the PR or CA soils that we observed under oxic conditions. Our results show that high rates of anoxic CO_2 production are largely driven by increases that occurred at 35 °C in these soils. This corresponded with a large increase in anoxic Fe(II) at 35 °C, suggesting that C oxidation coupled to dissimilatory microbial Fe reduction may be an important driver of anoxic CO_2 production under a warmer climate.

4.2. Effects of temperature change of microbial biomass C and MMQ

The MMQ is an important control on heterotrophic respiration (Xu et al., 2017). Here, the MMQ behaved differently in the two soil types under an anoxic headspace. Microbial biomass C concentrations in PR soils were significantly lower in the warmed treatment by the end of the experiment, particularly under anoxic conditions. This is likely driven by enzyme denaturation at higher temperature (Joergensen et al., 1990). However, MMQ Q_{10} values in both oxic and anoxic treatments increased relative to Q_{10} values taken from CO_2 production at the end of the experiment. These results suggest that a smaller pool of active microbes in the PR soils was able to respire at a much higher rate when subjected to elevated temperature between 26 and 35 °C. Specific ligninase-producing microbes are selected for at high temperatures to break down recalcitrant C (Chen et al., 2020), which may have contributed to the patterns observed. The increased rate of microbial respiration at higher temperatures corresponded to a significant increase in CH_4 production and higher Fe(II) concentrations. These results suggest that a combination of high temperature and low redox environments may lead to increased activity of anaerobic microbial communities, while simultaneously decreasing the size of the microbial biomass pool in the tropical soil. The significantly increased rates of CH_4 production at higher temperature suggest a "hot moments" mechanism (McClain et al., 2003), by which a significant portion of tropical soil CH_4 production may occur over short periods of high temperature conditions in low redox environments.

We observed different patterns in the temperate soils. The MBC concentrations in CA soils did not exhibit the same decrease between 26 and 35 °C by the end of the experiment. Consequently, MMQ Q_{10} values generally decreased relative to the Q_{10} values of CO_2 fluxes - the opposite of the result observed in PR soils. We also note that the increased temperature sensitivity of anaerobic metabolisms between 26 and 35 °C was not as large in CA soils as PR soils. While the increased temperature sensitivity of anaerobic metabolisms may be due to specialized microbial activity in PR soils, we speculate that it is more likely due to increase in microbial pool size in CA soils. Additionally, MMQ did not increase with increasing temperature in anoxic incubations ($Q_{10} < 1$). It is possible that the interaction of anoxia and high temperature may have an adverse effect on microbial respiration rates in this environment.

In PR soils we observed greater temperature sensitivity of MMQ between 26 and 35 °C in anoxic treatments relative to the oxic soils, whereas in CA soils we observed a lower Q_{10} value in anoxic samples. The different patterns observed in the PR and CA sites may result from the differences in the background climate regimes. The PR site experiences an average of 8 °C higher mean annual temperatures and almost 10 times as much rainfall annually as the CA site (Weaver and Murphy, 1990; Murphy et al., 2017; Hatala et al., 2012). These conditions likely favor specialized microbes that respire more efficiently under both higher temperatures and wet, anoxic conditions. Conversely, the more moderate conditions and higher background interannual variability at the temperate peatland site may favor more generalist microbial populations in these soils.

4.3. Coupling of methanogenesis and Fe reduction

Our results suggest a possible coupling of CH_4 production and Fe reduction. Methanogens and Fe-reducing microbes frequently use the same substrate, acetate, for metabolism (Roden and Wetzel, 1996). Methane fluxes and Fe(II) concentrations were positively correlated in both the PR and CA anoxic treatments. In PR soils, the HCl-extractable Fe was found to be almost completely in the reduced form of Fe(II), whereas Fe(II) composed approximately 60% of the HCl-extractable Fe measured in CA soils by the end of the experiment (Data not shown). High concentrations of poorly crystalline Fe mineral species have been found in drained CA Delta peatland soils, which may account for the remaining Fe(III) in this experiment (Anthony and Silver, 2020). Methane fluxes continued to increase through the course of the experiment in PR soil incubations, and also did so with high variability in CA soil incubations, even after Fe(II) plateaued in the second week. Iron-reducers may limit methanogenic access to acetate (Teh et al., 2008), and our results suggest that CH_4 emissions increased after reducible Fe(III) concentrations were exhausted. It is important to note that the presence of hydrogen in anaerobic incubations in this study may have facilitated hydrogenotrophic methanogenesis, so the correlation between CH_4 and Fe(II) is not necessarily due to competition for acetate. However, CH_4 fluxes measured at intermediate temperatures in this study were similar to those reported by McNicol and Silver (2014) using similar soils (CA peatland and PR tropical forest) and a pure N_2 atmosphere. Teh et al. (2008) also measured CH_4 fluxes from Puerto Rican rainforest soils under a pure N_2 headspace and reported a similar range of values found here across all treatments. That study interestingly found that Fe(III) reduction inhibited both acetoclastic and hydrogenotrophic methanogenesis (Teh et al., 2008).

4.4. Conclusion

We found that redox regimes played a significant role in the temperature sensitivity of biogeochemical processes in soils with variable redox conditions. The anaerobic biogeochemical processes analyzed here exhibited significantly increased temperature sensitivity at higher temperatures compared to lower temperatures, differing from consistent increases observed in oxic CO_2 production across temperature ranges. These patterns in the temperature sensitivity of redox biogeochemistry occurred in both the tropical and temperate sites. Anoxic MMQ measurements exhibited higher temperature sensitivity relative to oxic measurements in PR soils, but lower temperature sensitivity in CA soils, suggesting anaerobic microbial respiration is more efficient at high temperatures in the tropical environment. We suggest that increased anaerobic metabolisms at high temperature may be attributed to increased activity of a smaller proportion of the microbial biomass pool in the tropical soil but are more likely a function of microbial pool size in temperate environments. Additionally, we found that methanogenesis was correlated with Fe reduction in both systems. Our results suggest higher temperatures and increased extreme rainfall events that together can drive oxygen depletion in soils may interact to affect Fe, C, and GHG dynamics by increasing rates of anaerobic soil metabolisms differently than under oxic conditions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the many lab members and affiliates who helped with this experiment including Summer Ahmed, Tyler Anthony, Omar Gutierrez del Arroyo, Gisela Gonzalez, Hannah Marsh, Shane Russett, Uthara Vengrai, and Brian Yudkin. Additionally, Tibisay Perez, Benton Taylor, Calvin Heslop, and Wenying Liao provided insightful comments. This work was partially supported by the California Department of Water

Resources (award 4600011240). We thank the California Department of Water Resources and the Metropolitan Water District of Southern California for access to research sites. NRC was supported by the Rose Hills Independent Summer Undergraduate Research Fellowship. WLS was partially supported by McIntire Stennis grant CA-B-ECO-7673-MS as well as Breakthrough Strategies & Solutions, and the V. Kann Rasmussen, Oak Creek, Jewish Community, Northern Trust, and Trisons Foundations. This research was also partially supported by the National Science Foundation (grant nos. DEB-1457805, EAR-1331841, and DEB-0620910), and was part of the LUQ-LTER (DEB-1239764) and Luquillo CZO programs. Additional support was provided from DOE TES-DE-FOA-0000749.

References

Anthony, T.L., Silver, W.L., 2020. Mineralogical associations with soil carbon in managed wetland soils. Global Change Biology n/a. https://doi.org/10.1111/gcb.15309.

Baldock, J.A., Skjemstad, J.O., 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Organic Geochemistry 31, 697–710. https://doi.org/10.1016/S0146-6380(00)00049-8.

Beinroth, F.H., 1982. Some highly weathered soils of Puerto Rico, 1. Morphology, formation and classification. Characteristics, Genesis, and Classification of Strongly Weathered Soils of Puerto Rico 27, 1–73. https://doi.org/10.1016/0016-7061(82)90047-7.

Berhe, A.A., Suttle, K.B., Burton, S.D., Banfield, J.F., 2012. Contingency in the direction and mechanics of soil organic matter responses to increased rainfall. Plant and Soil 358, 371–383. https://doi.org/10.1007/s11104-012-1156-0.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17, 837–842. https://doi.org/10.1016/0038-0717(85)90144-0.

Buettner, S.W., Kramer, M.G., Chadwick, O.A., Thompson, A., 2014. Mobilization of colloidal carbon during iron reduction in basaltic soils. Geoderma 221–222, 139–145. https://doi.org/10.1016/j.geoderma.2014.01.012.

Canfield, D.E., Thamdrup, B., Hansen, J.W., 1993. The anaerobic degradation of organic matter in Danish coastal sediments: iron reduction, manganese reduction, and sulfate reduction. Geochimica et Cosmochimica Acta 57, 3867–3883. https://doi.org/10.1016/0016-7037(93)90340-3.

Chadwick, R., Good, P., Martin, G., Rowell, D.P., 2016. Large rainfall changes consistently projected over substantial areas of tropical land. Nature Climate Change 6, 177–181. https://doi.org/10.1038/nclimate2805.

Chen, J., Elsgaard, L., van Groenigen, K.J., Olesen, J.E., Liang, Z., Jiang, Y., Lærke, P.E., Zhang, Y., Luo, Y., Hungate, B.A., Sinsabaugh, R.L., Jørgensen, U., 2020. Soil carbon loss with warming: new evidence from carbon-degrading enzymes. Global Change Biology 26, 1944–1952. https://doi.org/10.1111/gcb.14986.

Coward, E.K., Thompson, A., Plante, A.F., 2018. Contrasting Fe speciation in two humid forest soils: insight into organomineral associations in redox-active environments. Geochimica et Cosmochimica Acta 238, 68–84. https://doi.org/10.1016/j.gca.2018.07.007.

Cui, M., Ma, A., Qi, H., Zhuang, X., Zhuang, G., Zhao, G., 2015. Warmer temperature accelerates methane emissions from the Zoige wetland on the Tibetan Plateau without changing methanogenic community composition. Scientific Reports 5, 11616. https://doi.org/10.1038/srep11616.

Davidson, E.A., Samanta, S., Caramori, S.S., Savage, K., 2012. The Dual Arrhenius and Michaelis–Menten kinetics model for decomposition of soil organic matter at hourly to seasonal time scales. Global Change Biology 18, 371–384. https://doi.org/10.1111/j.1365-2486.2011.02546.x.

DeAngelis, K.M., Silver, W.L., Thompson, A.W., Firestone, M.K., 2010. Microbial communities acclimate to recurring changes in soil redox potential status. Environmental Microbiology 12, 3137–3149. https://doi.org/10.1111/j.1462-2920.2010.02286.x.

Donat, M.G., Lowry, A.L., Alexander, L.V., O'Gorman, P.A., Maher, N., 2016. More extreme precipitation in the world's dry and wet regions. Nature Climate Change 6, 508–513. https://doi.org/10.1038/nclimate2941.

Fang, C., Moncrieff, J.B., 2001. The dependence of soil CO_2 efflux on temperature. Soil Biology and Biochemistry 33, 155–165. https://doi.org/10.1016/S0038-0717(00)00125-5.

Ginn, B., Meile, C., Wilmoth, J., Tang, Y., Thompson, A., 2017. Rapid iron reduction rates are stimulated by high-amplitude redox fluctuations in a tropical forest soil. Environmental Science & Technology 51, 3250–3259. https://doi.org/10.1021/acs.est.6b05709.

Greenwood, D.J., 1961. The effect of oxygen concentration on the decomposition of organic materials in soil. Plant and Soil 14, 360–376. https://doi.org/10.1007/BF01666294.

Gross, A., Pett-Ridge, J., Silver, W.L., 2018. Soil oxygen limits microbial phosphorus utilization in humid tropical forest soils. Soil Systems 2. https://doi.org/10.3390/soilsystems2040065.

Gutiérrez del Arroyo, O., Silver, W.L., 2018. Disentangling the long-term effects of disturbance on soil biogeochemistry in a wet tropical forest ecosystem. Global Change Biology 24, 1673–1684. https://doi.org/10.1111/gcb.14027.

Hall, S.J., Silver, W.L., 2015. Reducing conditions, reactive metals, and their interactions can explain spatial patterns of surface soil carbon in a humid tropical forest. Biogeochemistry 125, 149–165. https://doi.org/10.1007/s10533-015-0120-5.

Hatala, J.A., Detto, M., Sonnentag, O., Deverel, S.J., Verfaillie, J., Baldocchi, D.D., 2012. Greenhouse gas (CO2, CH4, H2O) fluxes from drained and flooded agricultural peatlands in the Sacramento-San Joaquin Delta. Agriculture, Ecosystems & Environment 150, 1–18. https://doi.org/10.1016/j.agee.2012.01.009.

Herndon, E.M., Mann, B.F., Roy Chowdhury, T., Yang, Z., Wullschleger, S.D., Graham, D., Liang, L., Gu, B., 2015. Pathways of anaerobic organic matter decomposition in tundra soils from Barrow, Alaska. Journal of Geophysical Research: Biogeosciences 120, 2345–2359. https://doi.org/10.1002/2015JG003147.

Intergovernmental Panel on Climate Change, 2014. Climate Change 2013 – the Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9781107415324.

Joergensen, R.G., Brookes, P.C., Jenkinson, D.S., 1990. Survival of the soil microbial biomass at elevated temperatures. Soil Biology and Biochemistry 22, 1129–1136. https://doi.org/10.1016/0038-0717(90)90039-3.

Kayranli, B., Scholz, M., Mustafa, A., Hedmark, Å., 2010. Carbon storage and fluxes within freshwater wetlands: a critical review. Wetlands 30, 111–124. https://doi.org/10.1007/s13157-009-0003-4.

Liptzin, D., Silver, W.L., 2009. Effects of carbon additions on iron reduction and phosphorus availability in a humid tropical forest soil. Soil Biology and Biochemistry 41, 1696–1702. https://doi.org/10.1016/j.soilbio.2009.05.013.

Lloyd, J., Taylor, J.A., 1994. On the temperature dependence of soil respiration. Functional Ecology 8, 315–323. https://doi.org/10.2307/2389824.

Lovley, D.R., Phillips, E.J., 1986. Organic matter mineralization with reduction of ferric iron in anaerobic sediments. Applied and Environmental Microbiology 51, 683–689. https://doi.org/10.1128/AEM.51.4.683-689.1986.

Lupascu, M., Wadham, J.L., Hornibrook, E.R.C., Pancost, R.D., 2012. Temperature sensitivity of methane production in the permafrost active layer at stordalen, Sweden: a comparison with non-permafrost northern wetlands. Arctic Antarctic and Alpine Research 44, 469–482. https://doi.org/10.1657/1938-4246-44.4.469.

McClain, M.E., Boyer, E.W., Dent, C.L., Gergel, S.E., Grimm, N.B., Groffman, P.M., Hart, S.C., Harvey, J.W., Johnston, C.A., Mayorga, E., McDowell, W.H., Pinay, G., 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6, 301–312. https://doi.org/10.1007/s10021-003-0161-9.

McNicol, G., Silver, W.L., 2014. Separate effects of flooding and anaerobiosis on soil greenhouse gas emissions and redox sensitive biogeochemistry. Journal of Geophysical Research: Biogeosciences 119, 557–566. https://doi.org/10.1002/2013JG002433.

Meier, J., Costa, R., Smalla, K., Boehrer, B., Wendt-Potthoff, K., 2005. Temperature dependence of Fe(III) and sulfate reduction rates and its effect on growth and composition of bacterial enrichments from an acidic pit lake neutralization experiment. Geobiology 3, 261–274. https://doi.org/10.1111/j.1472-4669.2006.00065.x.

Murphy, S.F., Stallard, R.F., Scholl, M.A., González, G., Torres-Sánchez, A.J., 2017. Reassessing rainfall in the Luquillo Mountains, Puerto Rico: local and global ecohydrological implications. PloS One 12, e0180987. https://doi.org/10.1371/journal.pone.0180987.

O'Connell, C.S., Ruan, L., Silver, W.L., 2018. Drought drives rapid shifts in tropical rainforest soil biogeochemistry and greenhouse gas emissions. Nature Communications 9, 1348. https://doi.org/10.1038/s41467-018-03352-3.

Peltoniemi, K., Laiho, R., Juottonen, H., Bodrossy, L., Kell, D.K., Minkkinen, K., Mäkiranta, P., Mehtätalo, L., Penttilä, T., Siljanen, H.M.P., Tuittila, E.-S., Tuomivirta, T., Fritze, H., 2016. Responses of methanogenic and methanotrophic communities to warming in varying moisture regimes of two boreal fens. Soil Biology and Biochemistry 97, 144–156. https://doi.org/10.1016/j.soilbio.2016.03.007.

Roden, E.E., Wetzel, R.G., 1996. Organic carbon oxidation and suppression of methane production by microbial Fe(III) oxide reduction in vegetated and unvegetated freshwater wetland sediments. Limnology & Oceanography 41, 1733–1748. https://doi.org/10.4319/lo.1996.41.8.1733.

Roy Chowdhury, T., Herndon, E.M., Phelps, T.J., Elias, D.A., Gu, B., Liang, L., Wullschleger, S.D., Graham, D.E., 2015. Stoichiometry and temperature sensitivity of methanogenesis and CO_2 production from saturated polygonal tundra in Barrow, Alaska. Global Change Biology 21, 722–737. https://doi.org/10.1111/gcb.12762.

Scanlon, D., Moore, T., 2000. Carbon dioxide production from peatland soil profiles: the influence of temperature, oxic/anoxic conditions and substrate. Soil Science 165. https://doi.org/10.1097/00010694-200002000-00006.

Schilling, K., Borch, T., Rhoades, C.C., Pallud, C.E., 2019. Temperature sensitivity of microbial Fe(III) reduction kinetics in subalpine wetland soils. Biogeochemistry 142, 19–35. https://doi.org/10.1007/s10533-018-0520-4.

Sierra, C.A., 2012. Temperature sensitivity of organic matter decomposition in the Arrhenius equation: some theoretical considerations. Biogeochemistry 108, 1–15. https://doi.org/10.1007/s10533-011-9596-9.

Silver, W.L., Lugo, A.E., Keller, M., 1999. Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils. Biogeochemistry 44, 301–328. https://doi.org/10.1007/BF00996995.

Stemmler, S.J., Berthelin, J., 2003. Microbial activity as a major factor in the mobilization of iron in the humid tropics. European Journal of Soil Science 54, 725–733. https://doi.org/10.1046/j.1351-0754.2003.0571.x.

Taylor, P.G., Cleveland, C.C., Wieder, W.R., Sullivan, B.W., Doughty, C.E., Dobrowski, S.Z., Townsend, A.R., 2017. Temperature and rainfall interact to control carbon

cycling in tropical forests. Ecology Letters 20, 779–788. https://doi.org/10.1111/ele.12765.

Teh, Y.A., Dubinsky, E.A., Silver, W.L., Carlson, C.M., 2008. Suppression of methanogenesis by dissimilatory Fe(III)-reducing bacteria in tropical rain forest soils: implications for ecosystem methane flux. Global Change Biology 14, 413–422. https://doi.org/10.1111/j.1365-2486.2007.01487.x.

Treat, C.C., Natali, S.M., Ernakovich, J., Iversen, C.M., Lupascu, M., McGuire, A.D., Norby, R.J., Roy Chowdhury, T., Richter, A., Šantrůčková, H., Schädel, C., Schuur, E. A.G., Sloan, V.L., Turetsky, M.R., Waldrop, M.P., 2015. A pan-Arctic synthesis of CH_4 and CO_2 production from anoxic soil incubations. Global Change Biology 21, 2787–2803. https://doi.org/10.1111/gcb.12875.

Turetsky, M.R., Treat, C.C., Waldrop, M.P., Waddington, J.M., Harden, J.W., McGuire, A. D., 2008. Short-term response of methane fluxes and methanogen activity to water table and soil warming manipulations in an Alaskan peatland. Journal of Geophysical Research: Biogeosciences 113. https://doi.org/10.1029/2007JG000496.

Viollier, E., Inglett, P.W., Hunter, K., Roychoudhury, A.N., Van Cappellen, P., 2000. The ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters. Applied Geochemistry 15, 785–790. https://doi.org/10.1016/S0883-2927(99)00097-9.

Voigt, C., Lamprecht, R.E., Marushchak, M.E., Lind, S.E., Novakovskiy, A., Aurela, M., Martikainen, P.J., Biasi, C., 2017. Warming of subarctic tundra increases emissions of all three important greenhouse gases – carbon dioxide, methane, and nitrous oxide. Global Change Biology 23, 3121–3138. https://doi.org/10.1111/gcb.13563.

Wang, X., Piao, S., Ciais, P., Janssens, I.A., Reichstein, M., Peng, S., Wang, T., 2010. Are ecological gradients in seasonal Q10 of soil respiration explained by climate or by vegetation seasonality? Soil Biology and Biochemistry 42, 1728–1734. https://doi.org/10.1016/j.soilbio.2010.06.008.

Weaver, P.L., Murphy, P.G., 1990. Forest structure and productivity in Puerto Rico's Luquillo mountains. Biotropica 22, 69–82. https://doi.org/10.2307/2388721.

White, J.R., Shannon, R.D., Weltzin, J.F., Pastor, J., Bridgham, S.D., 2008. Effects of soil warming and drying on methane cycling in a northern peatland mesocosm study. Journal of Geophysical Research: Biogeosciences 113. https://doi.org/10.1029/2007JG000609.

Xu, X., Schimel, J.P., Janssens, I.A., Song, X., Song, C., Yu, G., Sinsabaugh, R.L., Tang, D., Zhang, X., Thornton, PeterE., 2017. Global pattern and controls of soil microbial metabolic quotient. Ecological Monographs 87, 429–441. https://doi.org/10.1002/ecm.1258.

Yang, W.H., Liptzin, D., 2015. High potential for iron reduction in upland soils. Ecology 96, 2015–2020. https://doi.org/10.1890/14-2097.1.

Zheng, J., RoyChowdhury, T., Yang, Z., Gu, B., Wullschleger, S.D., Graham, D.E., 2018. Impacts of temperature and soil characteristics on methane production and oxidation in Arctic tundra. Biogeosciences 15, 6621–6635. https://doi.org/10.5194/bg-15-6621-2018.

Zhou, Z., Guo, C., Meng, H., 2013. Temperature sensitivity and basal rate of soil respiration and their determinants in temperate forests of north China. PloS One 8, e81793. https://doi.org/10.1371/journal.pone.0081793.