

Temperature and organic matter quantity drive CO₂ and CH₄ fluxes in isolated pools of non-perennial rivers

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Key Points:

- Isolated pools of non-perennial rivers have an important greenhouse gas emission potential under augmented temperature and organic matter
- Water temperature and organic matter had a synergistic effect on CH₄ fluxes from isolated pools during the late stages of incubation
- Water temperature and organic matter had positive individual effects on CO₂ fluxes from isolated pools

Abstract

Rivers are important contributors to global greenhouse gas (GHG) exchange with the atmosphere. However, much less is known about biogeochemical dynamics in rivers when they dry, particularly in isolated pools created by drying. Our objective was to examine the effects of water temperature and allochthonous organic matter (OM) quantity on carbon dioxide (CO₂) and methane (CH₄) fluxes in isolated pools. We used an automated analyzer to measure CO₂ and CH₄ from 36 mesocosms filled with sediments and water from a non-perennial river, with temperature (20, 25, 30 °C) and *Alnus glutinosa* leaf litter (2g, 5g, 10 g) manipulations in triplicate. We found positive individual effects of water temperature and OM quantity on CO₂ fluxes, and a synergistic effect of water temperature and OM on CH₄ fluxes during the late stages of the incubation. Given the increase in water temperature and OM inputs in rivers associated with climate change, our results indicate an associated increase in CO₂, and a disproportionate increase in CH₄ fluxes to the atmosphere, potentially contributing towards a positive climate feedback loop.

Plain Language Summary

Rivers play an important role in the exchange of greenhouse gases (GHGs) with the atmosphere. However, we have limited knowledge of how this exchange is altered when rivers dry, especially in the isolated pools formed during drying. In this study, we aimed to understand how the amount of leaf litter and the temperature of the water would effect the emission of carbon dioxide (CO₂) and methane (CH₄) from these isolated pools. We manipulated the leaf litter quantity and water temperature in 36 laboratory mesocosms containing riverine sediment and water. We found that higher water temperature and more leaf litter increased the release of CO₂. Moreover, the combined effect of these factors led to a disproportionate increase in the emissions of CH₄ during the later stages of the experiment. Our results suggest that as rivers dry more frequently with climate change and human impacts, the associated warmer water temperatures and increased leaf litter inputs could result in augmented emissions of CO₂ and CH₄, further contributing to climate change.

1 Introduction

River networks are hotspots of biogeochemical exchange with the atmosphere (Allen & Pavelsky, 2018; Battin et al., 2009; Cole, 2013; Raymond et al., 2013). Non-perennial rivers, i.e. those that periodically cease to flow, account for more than half of the length of the global river network (Messenger et al., 2021), but their contribution to biogeochemical cycles is yet understudied (Gómez-Gener et al., 2021; Marcé et al., 2019). Non-perennial rivers may represent a significant and likely increasing source of carbon dioxide (CO₂) and methane (CH₄), especially during sediment drying and rewetting phases (Keller et al., 2020; Paranaíba et al., 2021; von Schiller et al., 2014, 2019). However, much less is known about the contribution of isolated pools of non-perennial rivers in biogeochemical cycling, in particular greenhouse gas (GHG) production and emission. Isolated pools are difficult to detect and predict as they can be highly variable in space and time (Maswanganye et al., 2022), and they are largely not clearly reported in river flow records (Zimmer et al., 2020). Yet, prevailing conditions in these isolated pools suggest that they may have a high GHG production and emission potential (Chan et al., 2005).

Isolated pools are lentic, transitional environments between lotic and dry habitats of non-perennial rivers (Bonada et al., 2020; Datry et al., 2014). Rainwater inputs, bed morphology, evaporation, and substrate permeability play important roles in pool formation (Jocque et al., 2010; Siebers et al., 2020). The standing water in isolated pools, coupled with organic matter (OM) accumulation and high respiration rates, can create anoxic environments where anaerobic processes dominate (Day et al., 2019). Such anoxic conditions can promote methanogenesis and denitrification, leading to emissions of methane (CH₄) and nitrous oxide (N₂O) (Vazquez et al., 2011), two gases with high global warming potentials (Myhre et al., 2013). Therefore, it is critical to improve our understanding of the magnitudes and drivers of GHG fluxes in isolated pools to better understand the effects of drying on biogeochemical cycling.

The accumulation of OM and water temperature are likely among the key drivers of GHG production and emissions from isolated pools. Organic matter availability has been already reported as one of the most influential drivers of CO₂ (Datry et al., 2018; Keller et al., 2020; Luo & Zhou, 2010) and CH₄ (Serrano-Silva et al., 2014) fluxes in soils and freshwater sediments. High streambed sediment OM quantity and quality was associated with higher CO₂ and CH₄ fluxes in a laboratory incubation experiment (Romeijn et al., 2019). High concentrations of OM

frequently accumulate in isolated pools due to riparian vegetation inputs that, coupled with high rates of evaporation, lead to a high potential for CO₂ and CH₄ production (Granados et al., 2020).

Temperature is another important factor in determining the rates of respiration and other OM-dependant biogeochemical processes in various environments. For instance, soil microbial respiration rates have been shown to have a positive exponential relationship with soil temperature (Davidson & Janssens, 2006). Similarly, warming was found to increase microbial respiration rates from inland water sediments (Lü et al., 2023). The interactive effect between temperature and OM availability on CO₂ and CH₄ fluxes has been previously demonstrated in a laboratory incubation experiment using sediments from perennial rivers (Comer-Warner et al., 2018). The temperature sensitivity of CO₂ and CH₄ fluxes was dependent on substrate and OM content, with the largest response to increased temperature in fine, high OM content sediments (Comer-Warner et al., 2018). Yet, to date no study has examined these interactive effects on GHG fluxes in isolated pools of non-perennial rivers, where the microbial communities and their activity likely differ from those of perennial rivers due to the legacy effects of drying (Schreckinger et al., 2021).

Surface water temperatures and OM inputs to fluvial systems are likely to be altered in the face of global change, creating a positive feedback loop with climate change (Davidson & Janssens, 2006). Human activities have accelerated the cycling of carbon between terrestrial and aquatic systems and the atmosphere, wherein augmented terrestrial subsidies enhance carbon outgassing from inland waters (Regnier et al., 2022). Moreover, water temperature in rivers has been increasing in recent decades in parallel with increased air temperatures due to climate warming, and is expected to increase further under future climate scenarios (Michel et al., 2021; van Vliet et al., 2013). This simultaneous rise in water temperatures and OM inputs in non-perennial rivers may trigger an increase in CO₂ and CH₄ fluxes from isolated pools to the atmosphere.

The objective of this study was to determine how water temperature and allochthonous OM quantity influence CO₂ and CH₄ fluxes in simulated isolated pools of non-perennial rivers. We hypothesize that OM quantity and water temperature would have a synergistic effect on carbon emissions from isolated pools (Figure 1a). We predict that the rate of increase in carbon emissions with increasing temperature would be higher with greater OM content. We further

hypothesize that, over time, as low oxygen conditions develop in the mesocosms, we will observe a decline in CO₂ emission and an increase in CH₄ emission (Figure 1b). We tested these hypotheses using a controlled-laboratory mesocosm approach.

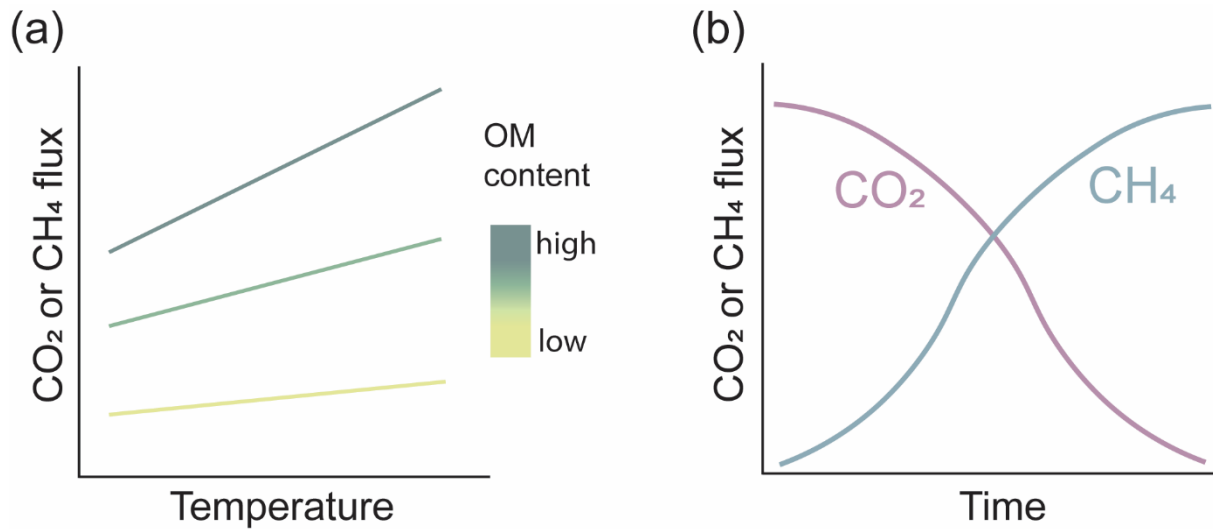


Figure 1. Predictions of the interactive effects of organic matter (OM) quantity and temperature on CO₂ and CH₄ fluxes (a); and the effect of time on CO₂ and CH₄ fluxes of isolated pools (b).

2 Materials and Methods

2.1 Study design

Mesocosms ($n = 36$) made of opaque PVC tubing (9.5 cm diameter, 30 cm height) with a removable lid fitted with an inlet and outlet tube connection to the gas analyser, were filled with riverine sediment and water from a non-perennial river and placed in a temperature-controlled laboratory (Figure 2). The laboratory air temperature was on average $25.36 \pm 0.27^\circ\text{C}$, as indicated by continuous datalogger air temperature measurements in the 12 hours prior to the experiment start. To simulate diurnal light conditions, the mesocosms were placed underneath programmed grow lights (RADIOMETRIX ® LED horticultural lighting system, Alpheus, France), programmed to be lit up from 7 am to 7 pm. We collected air-exposed riverine sediments from the top 15 cm of a dry intermittent reach of the Brévon Stream (45.96386, 5.442748), a tributary of the Albarine River, France in the summer of 2022, the day before our first mesocosm measurements on 2022-07-26. See Table S1 for a summary of the sediment physical characteristics. The location was chosen due to long-term knowledge of its drying history, confirmed by camera trap (Bushnell Core Dual Sensor, Overland Park, United States) observations taken bi-daily since June 2020 (two years prior to the start of this experiment), as well as the relatively fine sediment composition compared to the rest of the catchment. Based on the long-term camera trap data, we calculated a flow permanence of 18%, and the reach had been dry for the last 23 days before we sampled the sediment. We sieved the sediment to 5 mm to avoid any large stones or coarse organic matter in the mesocosms before adding, on average, $500.37 \text{ g} \pm 0.56$ of sediment to each mesocosm. To inoculate the mesocosms with a representative microbial decomposer community, we collected OM and natural foam along with stream water from 350 m upstream of where we collected the sediments, where water was flowing. We added 500 mL of this inoculum to each mesocosms after straining at $500 \mu\text{m}$, along with 1000 mL of groundwater to fill the mesocosms at the start of the experiment (“t0”) (Cornejo et al., 2020; López-Rojo et al., 2022).

Water temperature in the mesocosms (20, 25, 30°C) and the amount of OM (low: 2g; medium: 5g; and high: 10 g) were each manipulated, in triplicate, in a factorial design. The three mesocosms with only sediment (no added OM) served as controls. The temperature treatments were based on typical isolated pool temperatures in the Albarine River in France, which were

found to increased from 15°C to 25°C in the summer of 2011 (Datry, 2017). The highest temperature treatment, 30°C, was chosen as a temperature extreme, which may become a reality under increasing summer temperatures due to climate change. The OM treatments (which correspond to low: 282 g m⁻², medium: 705 g m⁻², and high: 1410 g m⁻²) were chosen as a range of leaf litter standing stocks representative of global estimates for dry reaches (min: 0 g m⁻², max: 7140 g m⁻², mean: 150.16 ± 555.91 g m⁻²) (Datry et al., 2018). Isolated pools may have even greater OM stocks than dry reaches, as depositional areas tend to have greater OM accumulation than riffles (Boulton & Lake, 1992). To control the temperature of the mesocosms, a commercial aquarium heater controlled by a Hobby Biotherm pro device (Dohse Aquaristik, Germany) was placed in each individual mesocosm. A datalogger (iButton datalogger, Maxim Integrated Products, USA) was placed in each mesocosm to record the actual temperature, which was on average 22.50 ± 0.54, 24.32 ± 0.74, and 27.26 ± 1.61 °C, per temperature treatment over the entire experiment period. For the OM treatments, freshly fallen *Alnus glutinosa* leaves were collected from a single upland stand in Chambéry, France in autumn 2020. The leaves were oven-dried at 48h at 70°C before being weighed (to 2, 5, or 10 g) and enclosed in 250 µm mesh bags and added to the mesocosms to measure the treatment effect on leaf litter decomposition. At the end of the incubation, the leaves were oven dried as above, and then burned at 550°C for 5h to estimate the ash-free dry mass (AFDM).

We additionally measured the temperature and dissolved oxygen (DO) concentration in each mesocosm (HQ series multiprobe, Hach Company, Loveland, CO, USA) at each measurement time from 72 hours after the start of the experiment, as well as the height (cm) from the water surface to the top of the mesocosm to account for the change in headspace volume due to evaporative water loss over time (Figure S1). Sediment OM content (%) of the pre-experiment sediment (composite samples) as well as the post-experiment sediment was measured by drying the sediment samples for 48h at 70°C before burning at 550°C for 5h. We measured sediment pH and conductivity from a 1:5 ratio of sediment to deionized water using the same multiprobe as above, using the average value from composite sediment samples taken in the field in June and September 2021.

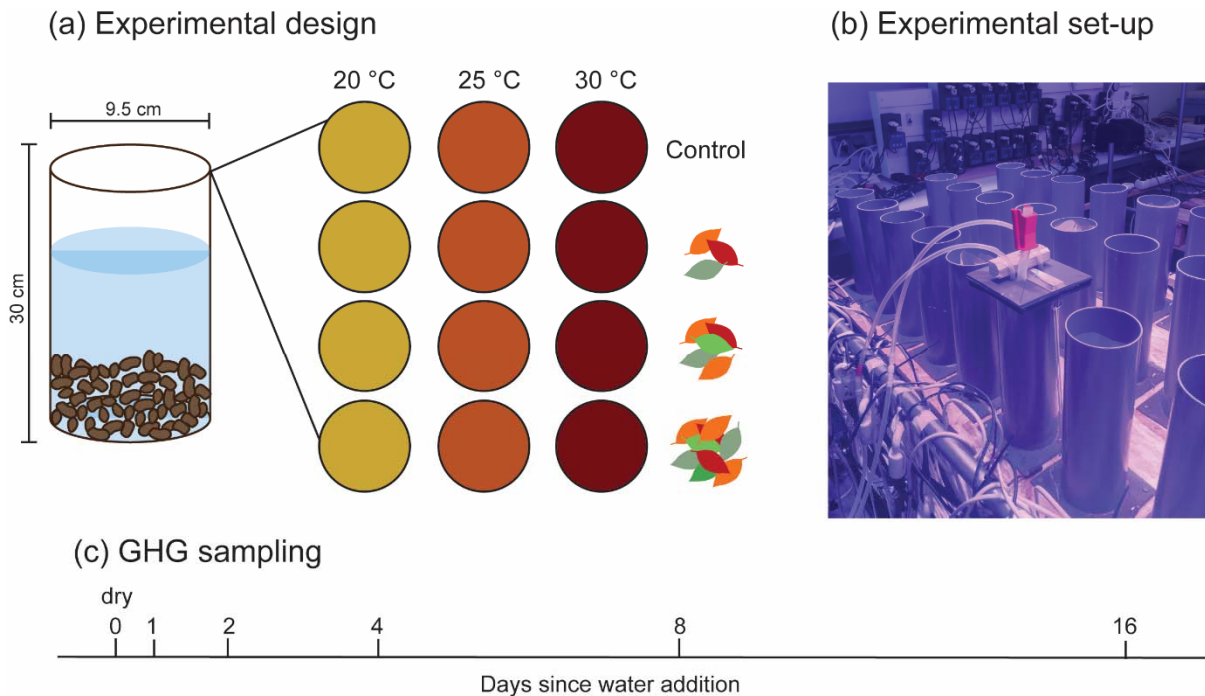


Figure 2. The experimental design of our laboratory experiment depicting the crossed temperature and organic matter quantity manipulations, in triplicate (a). A photograph of the experimental set up with 36 mesocosms made of opaque PVC and the lid used to measure headspace CO_2 and CH_4 concentrations (b). The greenhouse gas (GHG) sampling schedule, starting during a reference period when the sediments were dry, followed by t_0 immediately after adding water to the sediments, and then up to 16 days after water addition (c).

2.2 Greenhouse gas fluxes

We measured concentration the of CO_2 and CH_4 (ppm) in the headspace of the mesocosms during the reference period prior to water addition (“dry”) and immediately after wetting (“ t_0 ”) both on the first day of the experiment. We subsequently measured after 24 hours, 48 hours, 4 days, 8 days, and 16 days after the water addition. The experiment incubation time was informed by reported average isolated pool duration (21 days) in the Albarine river in the summer (Datry, 2017). A PVC foam-bottom lid was securely sealed on top of the mesocosm for GHG measurement. The lid was equipped with a vent tube (15 cm long, 1 cm diameter) to equalize the pressure between the mesocosm and the room for ~10 seconds before being closed for measurement (Hutchinson & Livingston, 2001). Prior to measurement, a small, 9V battery powered fan was positioned above the mesocosm to aerate the headspace, ensuring equivalent

ambient starting conditions across each mesocosm. The lid was connected to a portable GHG analyzer (MGGA, LGR-ICOS™ ABB-LGR, Cary, NC, USA) by transparent inlet and outlet tubes. GHG flux was then calculated based on the linear change in gas concentration over time from measurements recorded every second over an incubation period of 5 minutes, as follows:

$$F = \frac{dC}{dt} \times \frac{M \times V \times p}{A \times R \times T} \times 10^{-3}$$

Where F is the GHG flux (mg of CO₂-C or CH₄-C m⁻² h⁻¹), dC/dt is the linear slope of gas concentration over time (ppm h⁻¹), M is the molar mass (g mol⁻¹) of carbon, V is the mesocosm headspace volume (L), p is the assumed atmospheric pressure of 1 atm, A is the area of the chamber (m²), R is the universal gas constant (L atm K⁻¹ mol⁻¹), and T is the mean air temperature (K) of the laboratory. To convert the final flux from µg to mg, we multiplied the flux by a factor of 10⁻³. Each time series was visually inspected for quality control, where start and end times were trimmed to exclude any plateaus in concentrations.

2.3 Statistical analyses

To test for the treatment effects on CO₂ and CH₄ fluxes we used a model selection approach. We started with a saturated linear mixed model (LMM) using the *lmer* function from the ‘lme4’ R-package (Bates et al., 2015). Each model had CO₂ or CH₄ flux as the response variable, the categorical fixed factors of sampling day, water temperature, and OM quantity (and their interactions); dissolved oxygen; and the individual mesocosm column ID as the random effect. The LMMs excluded data from the reference period (dry) and initial wetting (t0), as the temperature manipulation was not yet in effect. In order to meet the assumptions of heteroscedasticity and normality of residuals for the LMMs, CO₂ and CH₄ flux were log transformed, with one and two outliers according to Cook’s Distance were removed from the CO₂ and CH₄ datasets, respectively. Using the *dredge* function from the ‘MuMIn’ R-package (Bartoń, 2019), we determined the most parsimonious model, as ranked by Akaike’s information criterion corrected for small sample sizes (AICc). To test the effects of temperature and OM on the leaf litter AFDM remaining we used a two-way Analysis of Variance (ANOVA), with the individual mesocosm column ID as a blocking factor. All mean values are denoted as mean ± the standard deviation. All statistical analyses were conducted using the statistical software R (version 4.3.0, R Core Team, 2023).

3 Results

3.1 Effects of temperature and organic matter quantity on greenhouse gas fluxes

We observed CO₂ efflux from all of the mesocosms, and CH₄ efflux on all but nine occasions, where we observed weak CH₄ uptake (once during the dry period, and eight times at t0). The average flux during the dry, reference period was 39.22 ± 14.02 mg CO₂-C m⁻² h⁻¹ and 0.024 ± 0.014 mg CH₄-C m⁻² h⁻¹. The average flux across all treatments and across the entire experiment period (excluding dry and t0 fluxes) was 78.30 ± 55.06 mg CO₂-C m⁻² h⁻¹ and 1.98 ± 4.53 mg CH₄-C m⁻² h⁻¹ (Table S2). The highest average flux rate occurred in the 30°C high OM treatments for both CO₂ (189.84 ± 54.00 mg CO₂-C m⁻² h⁻¹) and CH₄ (18.13 ± 44.13 mg CH₄-C m⁻² h⁻¹; Table S2).

The most parsimonious model explaining CO₂ fluxes included water temperature and OM quantity (and their interaction). The highest temperature treatment (30°C) had a positive effect on CO₂ fluxes (LMM; $\beta = 0.51$, $p = <0.001$; Table 1). Organic matter had a positive effect on CO₂ fluxes at all treatment levels compared to the control (Table 1). The addition of OM (all treatments averaged) resulted in a CO₂ flux on average 5 times higher than the control (Figure S2). We did not find an interactive effect of temperature and OM quantity on CO₂ fluxes (Table 1, Figure 3a).

The most parsimonious model explaining CH₄ fluxes included water temperature, OM quantity, and time (and their interaction). In contrast to CO₂, there was no individual effect of temperature or OM on CH₄ fluxes (Table 1), except on day 16 where CO₂ was significantly higher at the high and medium OM treatments. Moreover, we did not find an interactive effect of temperature and OM quantity of CH₄ fluxes (Table 1, Figure 3b). However, when in interaction with date, we found interactive effects of temperature and OM on days 8 and 16 of the experiment (Table 1, Figure 4 d-f). The addition of OM resulted in a CH₄ flux on average 182 times higher than the control with no OM (Figure S2).

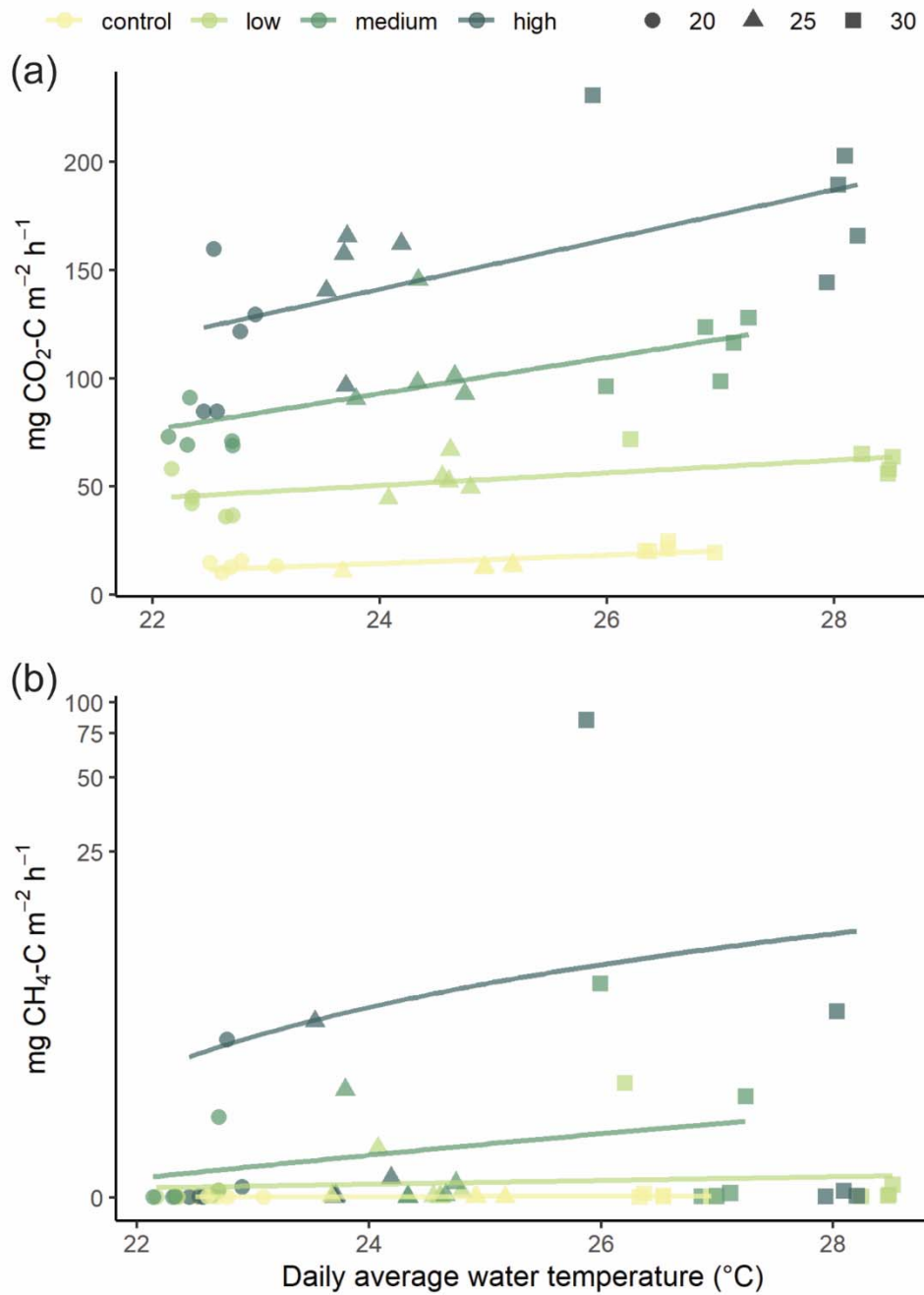


Figure 3. The interactive effect of daily average actual measured water temperature (°C) and *Alnus glutinosa* leaf litter quantity (control = 0g, low = 2 g, medium = 5 g, high = 10 g) on (a) CO₂ and (b) CH₄ flux from mesocosms (excluding dry and t0 flux values). See Figure S3 for the time series of actual measured water temperatures by treatment.

3.2 Temporal dynamics of greenhouse gas fluxes

Date was included in the most parsimonious model for CH₄ fluxes, but not for CO₂ fluxes (Table 1, Table S3). For CH₄, we did not find individual effects of the sampling dates, but rather interactive effects between date and the other treatments. Firstly, the interaction of day 16 and OM (medium and high) had a positive effect on CH₄ fluxes (Table 1). Secondly, day 8 and 16 had a positive interactive effect with OM and temperature on CH₄ fluxes (Table 2). If we consider the dry reference period and t0 (not included in our LMMs), we can see a general pattern of a peak in CO₂ 24h after water was added, a small decline to 2 days, before stabilization. The lowest average flux was measured at t0, immediately after water was added ($25.81 \pm 11.45 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$), and the highest average flux was measured on the fourth day of the experiment ($88.24 \pm 65.02 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$; Figure 4a-c). For CH₄, the lowest average flux was also at t0 ($0.48 \pm 0.58 \text{ } \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$), and the highest was recorded on the 16th day of the experiment ($9240.86 \pm 29,498.55 \text{ } \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$; Figure 4d-f). We were unable to fully investigate the temporal dynamics of DO concentrations, as our first measurement was at 3 days after water was added to the mesocosms (Figure S4). However, we detected hypoxic conditions, approaching anoxic conditions ($\text{DO} < 0.27 \text{ mg L}^{-1}$) in all of the OM addition treatments since day 3 for the duration of the experiment (Figure S4). Here, we defined hypoxia as DO concentrations $< 2 \text{ mg L}^{-1}$ and anoxia as the complete absence of oxygen (Pardo & García, 2016). With our available data, we found negative effect of OM (all treatment levels) on DO, and a significant decline in DO on the last day compared to the third day (Figure S4, Table S4).

3.3 Effects of temperature and organic matter quantity on leaf litter mass loss

We did not find an effect of temperature or OM quantity individually, nor in interaction on the leaf litter AFDM remaining (Table 2; Figure 5). However, we observe a trend in decreasing AFDM remaining at the 30°C temperature treatment with increasing OM (Figure 5). An approximate mass balance calculation over the entire incubation period estimated that 68% of the organic carbon mass fraction of the leaf litter loss was out-gassed in CO₂ equivalents (CO₂ e), with 51% from CO₂ and 16% from CH₄ (CO₂ e).

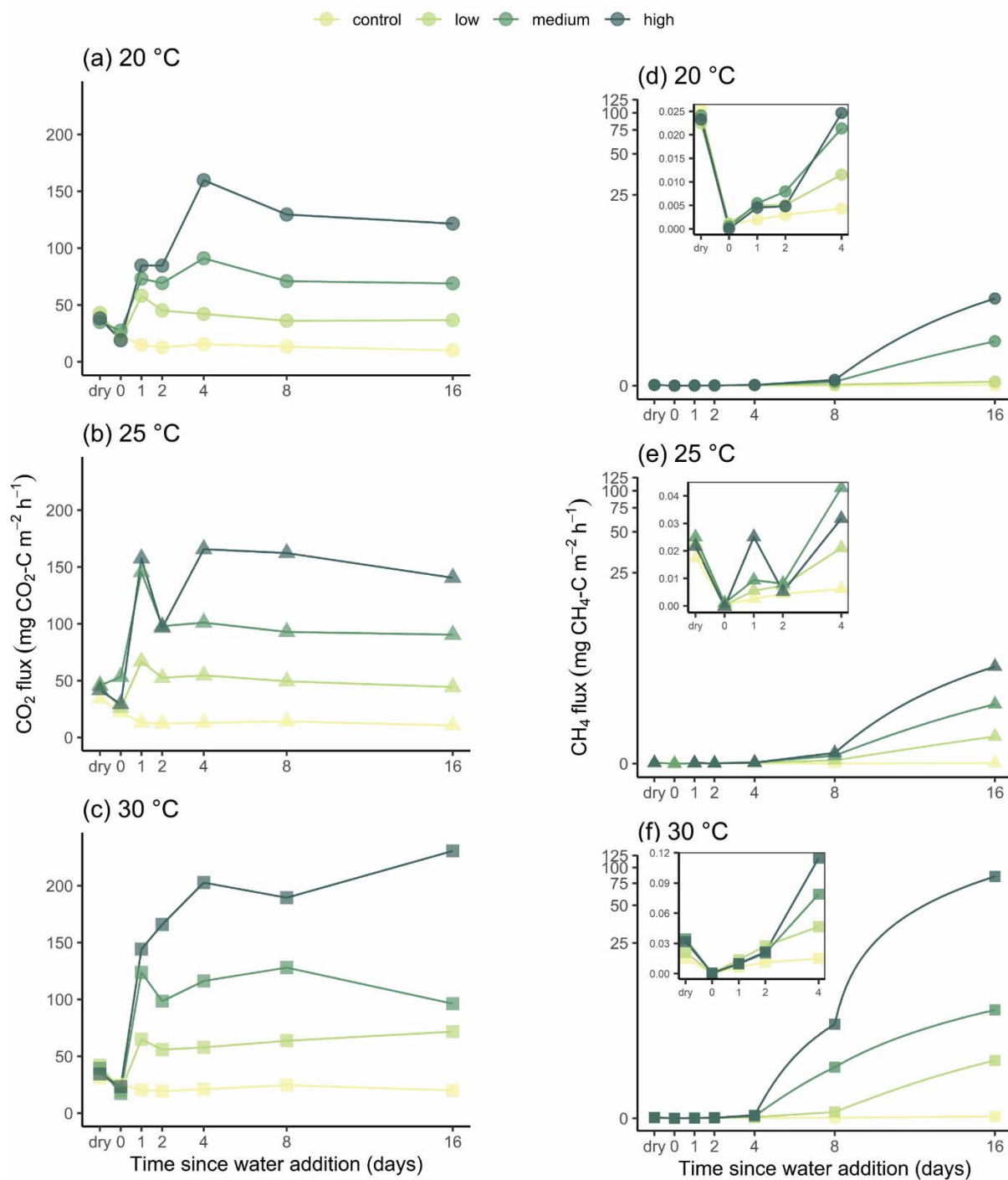


Figure 4. Time series of CO₂ (a, b, c) and CH₄ (d, e, f) flux from mesocosms before (time since wetting = ‘dry’) and following their inundation (time since wetting = 0 days) across crossed manipulations of *Alnus glutinosa* leaf litter quantity (control = 0g, low = 2 g, medium = 5 g, high = 10 g) and temperatures. The inset plots show a magnified view of CH₄ flux until 4 days after wetting.

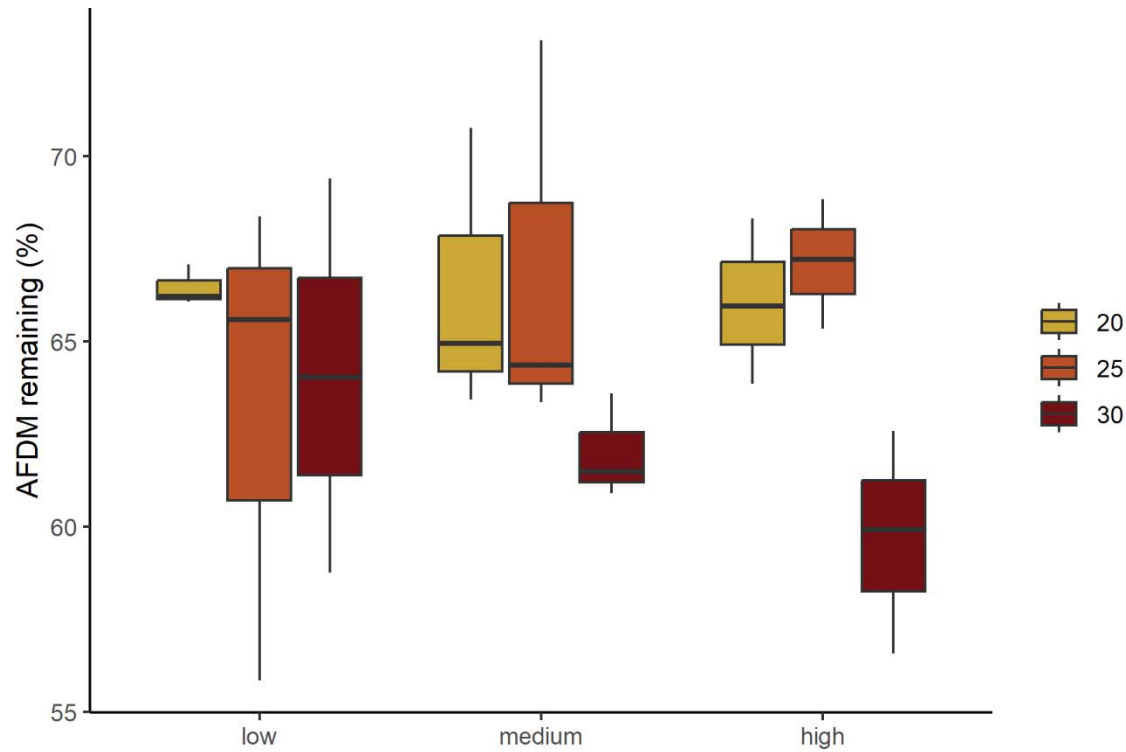


Figure 5. *Alnus glutinosa* leaf litter ash-free dry mass (AFDM) remaining (%) at the end of the incubation in simulated isolated pools under crossed temperature (20, 25, 30 °C) and leaf litter quantity (low = 2 g, medium = 5 g, high = 10 g). Note that each boxplot represents $n = 3$ replicates.

Table 1. The model estimate (β), standard error (SE) and p-value (p) of the fixed effects from the top linear mixed model (LMM), ranked by AIC, explaining the effects of organic matter (OM) quantity, water temperature (Temp; °C), and time (t; days since wetting) on CO₂ and CH₄ fluxes from mesocosms simulating isolated pools. Note that this analysis excludes data from the reference period (dry) and initial wetting (t0). CO₂ and CH₄ flux were $\log(x_{\min}+1)$ transformed, and one and two outliers were removed from the CO₂ and CH₄ datasets, respectively, based on Cook's Distance. Statistical significance of a model term is indicated in bold. Due to the large number ($n = 59$) of terms in the LMM for CH₄, here we only show the common terms with CO₂, and any significant terms. For the full model output see the Supplementary Materials (Table S3).

Marginal R ²	CO ₂			CH ₄		
	β	SE	p	β	SE	p
Temp 25	0.04	0.12	0.74	<0.001	0.13	1.00
Temp 30	0.51	0.11	<0.001	0.005	0.13	0.97
OM_{low}	1.23	0.11	<0.001	0.003	0.13	0.98
OM_{med}	1.76	0.11	<0.001	0.003	0.13	0.98
OM_{high}	2.18	0.11	<0.001	0.003	0.13	0.98
Temp 25 * OM _{low}	0.17	0.16	0.31	<-0.001	0.18	1.00
Temp 30 * OM _{low}	-0.14	0.16	0.40	0.004	0.18	0.98
Temp 25 * OM _{med}	0.31	0.16	0.07	0.003	0.18	0.99
Temp 30 * OM _{med}	-0.10	0.16	0.56	<-0.001	0.18	1.00
Temp 25 * OM _{high}	0.20	0.16	0.24	0.02	0.18	0.92
Temp 30 * OM _{high}	-0.02	0.16	0.92	<0.001	0.18	1.00
t16 * OM_{med}	-	-	-	0.95	0.17	<0.001
t16 * OM_{high}	-	-	-	1.57	0.17	<0.001
t8 * OM_{med} * Temp 30	-	-	-	1.00	0.24	<0.001
t8 * OM_{high} * Temp 30	-	-	-	1.44	0.24	<0.001
t16 * OM_{low} * Temp 25	-	-	-	0.51	0.24	0.04
t16 * OM_{low} * Temp 30	-	-	-	1.06	0.24	<0.001
t16 * OM_{med} * Temp 30	-	-	-	1.06	0.24	<0.001
t16 * OM_{high} * Temp 30	-	-	-	2.93	0.27	<0.001

289

290

Table 2. The model sum of squares (*SS*), degrees of freedom (*SE*), F-value (*F*), and p-value (*p*) of the fixed effects from the two-way Analysis of Variance (ANOVA) explaining variation in *Alnus glutinosa* leaf litter ash-free dry mass (AFDM) remaining (%) after 16 days in mesocosms simulating isolated pools.

	<i>SS</i>	<i>df</i>	<i>F</i>	<i>p</i>
Temperature	79.09	2	2.69	0.10
OM	5.52	2	0.19	0.83
Temperature * OM	54.45	4	0.93	0.47
Residuals	250.09	17		

4 Discussion

Our results demonstrate that isolated pools have an important GHG emission potential, especially under augmented temperature and OM, as our reported carbon fluxes were generally higher than reported values for other inland waters, with the exception of lotic waters (Table S5). The fluxes during the dry reference period were on average lower than when the sediments were inundated, demonstrating the importance of isolated pools as hotspots of carbon emissions in non-perennial rivers. Our CO₂ and CH₄ fluxes were in the range of reported values for the field studies of isolated pools and temporary ponds (Table S5). There are limited field studies examining CO₂ and CH₄ fluxes from isolated pools, likely due to the difficulty to capture pools given their highly ephemeral nature (Maswanganye et al., 2022). In one of the only field studies to measure CO₂ and CH₄ from isolated pools in a Mediterranean intermittent river network, pools had relatively low CO₂ and CH₄ flux rates (8.61 mg CO₂-C m⁻² h⁻¹ and 0.05 mg CH₄-C m⁻² h⁻¹) (Gómez-Gener et al., 2015). There was a strong effect of physical limitation on CO₂ efflux, as CO₂ fluxes from isolated pools were the lowest, when compared to lotic waters, dry sediments, and reservoirs, despite having the highest dissolved CO₂ concentrations (Gómez-Gener et al., 2015). Moreover, the unexpectedly low CH₄ flux from isolated pools, despite the high water residence times and optimal redox conditions for methanogenic activity, was attributed to low OM availability (Gómez-Gener et al., 2015). We expected to underestimate emissions compared to natural isolated pools because we did not account for carbon inputs from lateral water exchange (Yu et al., 2022) nor the effects of mixing due to strong wind or rainstorms (Reverey et al., 2016). However, pools can become increasingly disconnected from lateral hydrologic connections over time (Gómez-Gener et al., 2020). Thus, lateral inputs may present a small source of carbon emissions from isolated pools in non-perennial rivers, especially at later stages of drying (Gómez-Gener et al., 2020). Our study underlines the usefulness of using a mesocosm approach to examine the biogeochemistry of isolated pools in non-perennial rivers, particularly in disentangling the drivers of GHG fluxes.

4.1 Effects of temperature and organic matter quantity on greenhouse gas fluxes

Our results provide partial support for our first hypothesis of a synergistic effect of temperature and OM on GHG fluxes, as a synergistic effect was only observed for CH₄ on our last two sampling days. A review study showed that the temperature dependence of

methanogenesis is consistently reported in the literature from the microbial to the ecosystem scale (Yvon-Durocher et al., 2014). Methanogenesis was found to be disproportionately more sensitive to long-term experimental warming than methanotrophy in artificial ponds (Zhu et al., 2020). Moreover, the temperature dependence of CH₄ emission is estimated to be considerably higher than that of CO₂ (Yvon-Durocher et al., 2014). We did not observe a similar synergistic effect on CO₂ fluxes, but rather positive individual effects of temperature and OM content. In a similar study, CO₂ fluxes from lab incubated streambed sediments increased significantly with increasing temperature (Comer-Warner et al., 2018). An interactive effect of temperature with sediment substrate type (texture and OM content) was detected, with higher CO₂ flux from finer, higher OM content sediments than medium and coarse, lower OM content sediments (Comer-Warner et al., 2018). Their study aimed to simulate surface water with a 5h incubation, whereas in our study we measured over 16 days, simulating realistic isolated pool conditions (Datry, 2017). Thus, the lack of synergistic effect on CO₂ here may be related to the rapid depletion of oxygen in the high temperature and high OM mesocosms, resulting in relatively higher production of CH₄ than CO₂.

The significant contribution of leaf litter decomposition to the total carbon efflux from isolated pools is highlighted by the control treatment with no OM which consistently had the lowest average CO₂ and CH₄ flux. In fact, the addition of OM (low to high treatments combined) to the sediments resulted in a 5x and 182x increase in CO₂ and CH₄ fluxes, respectively, compared to the control with sediments alone. These results are in contrast with studies of respiration pulses from rewetting of non-perennial rivers. In a global research effort, respiration rates from rewetted sediments were higher, 417 mg CO₂-C m⁻² h⁻¹ (von Schiller et al., 2019) than the rates from rewetted leaf litter alone, 37 mg CO₂-C m⁻² h⁻¹ (Datry et al., 2018), from the same sites. One possible reason for this discrepancy could be that we measured carbon fluxes from sediments and OM together, wherein the sediments may have served as an inoculum of bacteria and fungi for the processing of the OM (Cornut et al., 2010). It may also be explained by the disparate dynamics between sediment rewetting which is a short-term phenomenon, while in isolated pools, there is more time for microbial activity to resume. In addition, in the Datry et al. (2018) study, the leaves were collected from dry streambeds, and therefore may have been subject to preconditioning effects of rain and solar radiation, which reduced their quality and biodegradability when compared to the leaves in our experiment (del Campo et al., 2019). As

such, our findings underline the particular importance of leaf litter in driving carbon emissions from isolated pools.

4.2 Temporal dynamics of greenhouse gas fluxes

We only found partial support of our second hypothesis that CO₂ fluxes would decline and CH₄ fluxes would increase over time. We found no effect of sampling date on CO₂ fluxes and an interactive effect of temperature and OM on CH₄ fluxes days 8 and 16. A decline in dissolved oxygen concentrations may have been associated with this increase in CH₄ fluxes near the end of the experiment, as we found lower DO concentrations on the last day of the experiment compared to earliest DO measurement. However, we had hypoxic conditions, approaching anoxic conditions in all of the OM addition treatments since day 3 for the duration of the experiment, which should have consistently promoted methanogenesis. Alternatively, the significant evaporation that occurred by days 8 and 16 may also explain the increase in CH₄ fluxes as the evaporation may have impacted the physico-chemical characteristics of the water. For example, dissolved organic matter and dissolved nutrient concentrations in natural isolated pools were the greatest in pools with high evaporative water loss (Fellman et al., 2011). Another possible explanation for this temporal trend in CH₄ could be a lag response time of methanogenic archaea after low oxygen conditions are created following inundation (Watanabe et al., 2007). For example, CH₄ production started six days following the inundation of air-dried soils from rice paddy fields (Watanabe et al., 2007). Similarly, we only started to observe appreciable CH₄ fluxes after four days. The temporal trend in CH₄ fluxes that we observed indicates that pools with a longer duration of one to two weeks are more likely to be significant sources of CH₄ than highly ephemeral pools that last for only a few days.

The absence of a significant temporal trend in CO₂ fluxes may have been because some of the CH₄ produced was converted to CO₂ through methanotrophy, potentially in aerobic microsites, as has been observed in soils (Khalil & Baggs, 2005; Levy-Booth et al., 2019) and streambed sediments (Shelley et al., 2015). Additionally, in the absence of oxygen, CO₂ can be produced by various fermentation pathways (Fairbairn et al., 2023). The lack of a decrease in CO₂ over time may also be related to the OM availability which was not limiting, as we had > 50% AFDM remaining in all treatments at the end of the experiment. In a field study of isolated pools along a non-perennial river, CO₂ production in isolated pools significantly increased with

time since disconnected, and DO concentrations significantly declined (Granados et al., 2020). This positive response of CO₂ production was observed immediately upon temporary pool formation, while DO concentrations had a delayed response, with a decline starting at seven days after disconnection (Granados et al., 2020). However, despite the high CO₂ production, there may be relatively low emissions due to limited gas exchange in stagnant waters (Gómez-Gener et al., 2015; Granados et al., 2020). More research into microbial community and microsite dynamics is necessary to improve our understanding of the temporal dynamics of CO₂ in isolated pools.

5 Conclusions

We found positive individual effects of water temperature and OM on CO₂ fluxes, and a synergistic effect of water temperature and OM on CH₄ fluxes during the late stages of the incubation. Understanding the influence of factors such as temperature and OM on GHG fluxes from isolated pools, which coincide with drying events in river networks, is of increasing relevance given that global change is likely to exacerbate the global extent of non-perennial rivers (Datry et al., 2023). Historically perennial rivers may dry due to the combined effects of water abstraction and climate change (Döll & Schmied, 2012). As such, future studies should examine the functional response of sediments contrasting natural vs. anthropogenic non-perennial rivers. Microbial communities in naturally non-perennial rivers may be better adapted to cope with drying than those in anthropogenic non-perennial rivers (Reverey et al., 2016; Schreckinger et al., 2021). An additional avenue that we did not consider in our study is the quality of organic matter in addition to the quantity, which plays an important role in CO₂ and CH₄ fluxes from streambed sediments (Romeijn et al., 2019). Moreover, examining the microbial communities responsible for OM processing in isolated pools would give us a greater insight into the mechanisms controlling GHG fluxes (Arora-Williams et al., 2022).

The dry phases of non-perennial inland waters are not considered in current estimates of global GHG fluxes from freshwaters (Drake et al., 2018; Marcé et al., 2019; Raymond et al., 2013). Here, we show that isolated pools of non-perennial rivers, simulated under controlled laboratory conditions, have the potential to contribute significant CO₂ and CH₄ emissions to the atmosphere under augmented temperature and OM. Given these findings, in addition to what has been reported during sediment drying (Keller et al., 2020; Paranaíba et al., 2021) and rewetting

(Datry et al. 2018), we strongly advocate for the inclusion of GHG fluxes of non-perennial rivers in global GHG budgets.

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Author Contributions

Conceptualization: TS, TD, VC. Funding acquisition: VC and TD. Investigation: TS and AA. Analysis: TS. Writing (original draft): TS. Writing (review and editing): TS, TD, VC, FC, NLR, AA.

Open Research

Data and programming code to replicate analyses and figures are available at <https://github.com/TeresaSilverthorn/Mesocosm>

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