

1
2 **Temperature and organic matter quantity drive CO₂ and CH₄ fluxes in isolated pools**
3 **of non-perennial rivers**
4

5 **T. Silverthorn¹, A. Azougui¹, N. López-Rojo¹, F. Colas², V. Chanudet³, and T. Datry¹**

6
7 ¹ National Institute for Agriculture, Food, and Environment (INRAE), RiverLy Research Unit,
8 Centre de Lyon-Grenoble Auvergne-Rhône-Alpes, Villeurbanne, France.

9 ² Université Claude Bernard Lyon 1, CNRS, ENTPE, Villeurbanne, France.

10 ³ Électricité de France (EDF), Hydro Engineering Centre, Environmental and Social Dept.,
11 HYNES (Irstea – EDF R&D), Savoie Technolac, La Motte Servolex, France.

12
13 Corresponding author: Teresa Silverthorn (teresa.silverthorn@gmail.com)
14
15

16 **Key Points:**

- 17 • Isolated pools of non-perennial rivers have an important greenhouse gas emission
18 potential under augmented temperature and organic matter
- 19 • Water temperature and organic matter had a synergistic effect on CH₄ fluxes from
20 isolated pools during the late stages of incubation
- 21 • Water temperature and organic matter had positive individual effects on CO₂ fluxes from
22 isolated pools
23

24 Abstract

25 Rivers are important contributors to global greenhouse gas (GHG) exchange with the
26 atmosphere. However, much less is known about biogeochemical dynamics in rivers when they
27 dry, particularly in isolated pools created by drying. Our objective was to examine the effects of
28 water temperature and allochthonous organic matter (OM) quantity on carbon dioxide (CO₂) and
29 methane (CH₄) fluxes in isolated pools. We used an automated analyzer to measure CO₂ and CH₄
30 from 36 mesocosms filled with sediments and water from a non-perennial river, with temperature
31 (20, 25, 30 °C) and *Alnus glutinosa* leaf litter (2g, 5g, 10 g) manipulations in triplicate. We found
32 positive individual effects of water temperature and OM quantity on CO₂ fluxes, and a
33 synergistic effect of water temperature and OM on CH₄ fluxes during the late stages of the
34 incubation. Given the increase in water temperature and OM inputs in rivers associated with
35 climate change, our results indicate an associated increase in CO₂, and a disproportionate
36 increase in CH₄ fluxes to the atmosphere, potentially contributing towards a positive climate
37 feedback loop.

38 Plain Language Summary

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

51 **1 Introduction**

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

65 Isolated pools are lentic, transitional environments between lotic and dry habitats of non-
66 perennial rivers (Bonada et al., 2020; Datry et al., 2014). Rainwater inputs, bed morphology,
67 evaporation, and substrate permeability play important roles in pool formation (Jocque et al.,
68 2010; Siebers et al., 2020). The standing water in isolated pools, coupled with organic matter
69 (OM) accumulation and high respiration rates, can create anoxic environments where anaerobic
70 processes dominate (Day et al., 2019). Such anoxic conditions can promote methanogenesis and
71 denitrification, leading to emissions of methane (CH₄) and nitrous oxide (N₂O) (Vazquez et al.,
72 2011), two gases with high global warming potentials (Myhre et al., 2013). Therefore, it is
73 critical to improve our understanding of the magnitudes and drivers of GHG fluxes in isolated
74 pools to better understand the effects of drying on biogeochemical cycling.

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

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

83 Temperature is another important factor in determining the rates of respiration and other
84 OM-dependant biogeochemical processes in various environments. For instance, soil microbial
85 respiration rates have been shown to have a positive exponential relationship with soil
86 temperature (Davidson & Janssens, 2006). Similarly, warming was found to increase microbial
87 respiration rates from inland water sediments (Lü et al., 2023). The interactive effect between
88 temperature and OM availability on CO₂ and CH₄ fluxes has been previously demonstrated in a
89 laboratory incubation experiment using sediments from perennial rivers (Comer-Warner et al.,
90 2018). The temperature sensitivity of CO₂ and CH₄ fluxes was dependent on substrate and OM
91 content, with the largest response to increased temperature in fine, high OM content sediments
92 (Comer-Warner et al., 2018). Yet, to date no study has examined these interactive effects on
93 GHG fluxes in isolated pools of non-perennial rivers, where the microbial communities and their
94 activity likely differ from those of perennial rivers due to the legacy effects of drying
95 (Schreckinger et al., 2021).

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

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

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

114

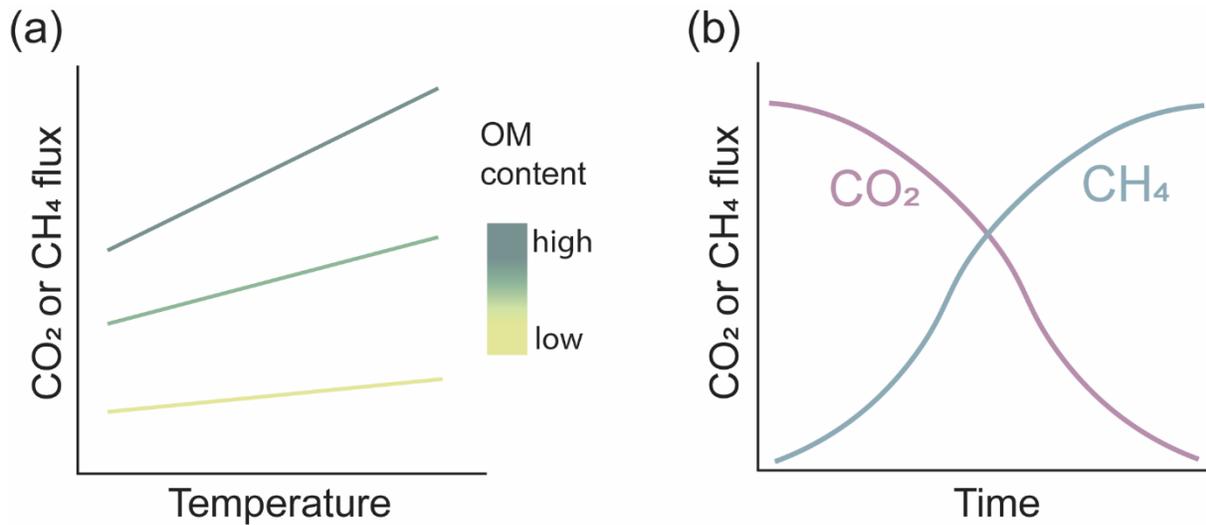


Figure 1. Predictions of the interactive effects of organic matter (OM) quantity and temperature on CO₂ and CH₄ fluxes (a); and the effect of time on CO₂ and CH₄ fluxes of isolated pools (b).

115

116

117 2 Materials and Methods

118 2.1 Study design

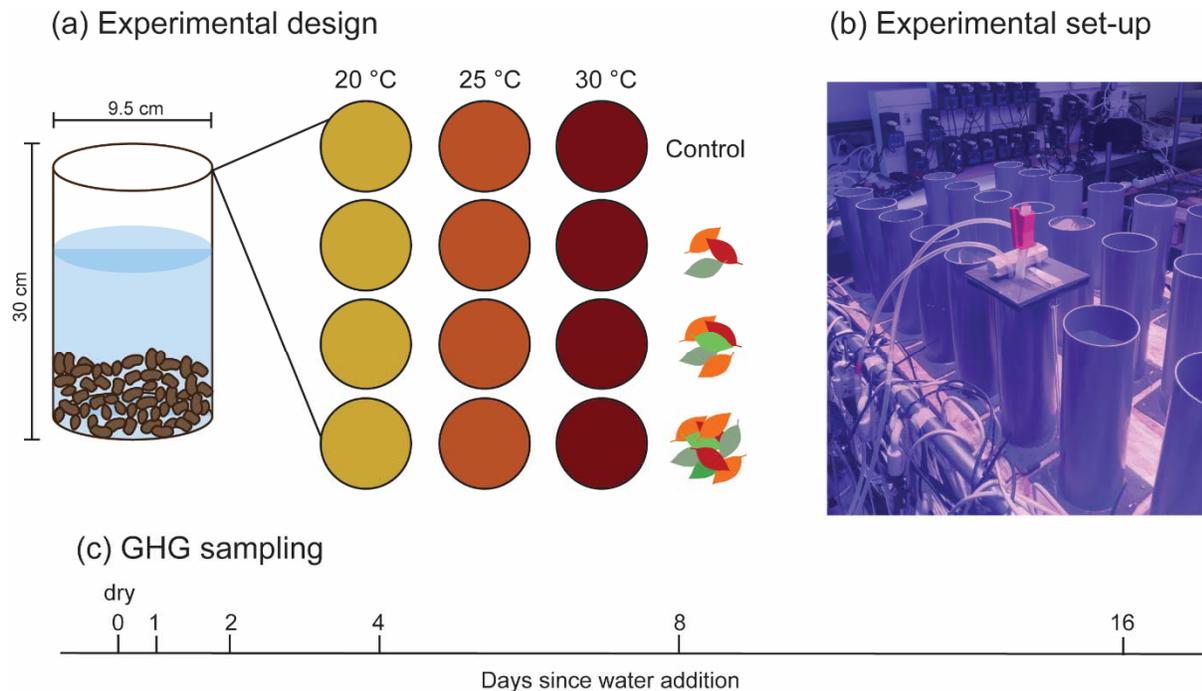
119 Mesocosms ($n = 36$) made of opaque PVC tubing (9.5 cm diameter, 30 cm height) with a
120 removable lid fitted with an inlet and outlet tube connection to the gas analyser, were filled with
121 riverine sediment and water from a non-perennial river and placed in a temperature-controlled
122 laboratory (Figure 2). The laboratory air temperature was on average $25.36 \pm 0.27^\circ\text{C}$, as
123 indicated by continuous datalogger air temperature measurements in the 12 hours prior to the
124 experiment start. To simulate diurnal light conditions, the mesocosm were placed underneath
125 programmed grow lights (RADIOMETRIX® LED horticultural lighting system, Alpheus,
126 France), programmed to be lit up from 7 am to 7 pm. We collected air-exposed riverine
127 sediments from the top 15 cm of a dry intermittent reach of the Brévon Stream (45.96386,
128 5.442748), a tributary of the Albarine River, France in the summer of 2022, the day before our
129 first mesocosm measurements on 2022-07-26. See Table S1 for a summary of the sediment
130 physical characteristics. The location was chosen due to long-term knowledge of its drying
131 history, confirmed by camera trap (Bushnell Core Dual Sensor, Overland Park, United States)
132 observations taken bi-daily since June 2020 (two years prior to the start of this experiment), as
133 well as the relatively fine sediment composition compared to the rest of the catchment. Based on
134 the long-term camera trap data, we calculated a flow permanence of 18%, and the reach had been
135 dry for the last 23 days before we sampled the sediment. We sieved the sediment to 5 mm to
136 avoid any large stones or coarse organic matter in the mesocosms before adding, on average,
137 $500.37 \text{ g} \pm 0.56$ of sediment to each mesocosm. To inoculate the mesocosms with a
138 representative microbial decomposer community, we collected OM and natural foam along with
139 stream water from 350 m upstream of where we collected the sediments, where water was
140 flowing. We added 500 mL of this inoculum to each mesocosms after straining at $500 \mu\text{m}$, along
141 with 1000 mL of groundwater to fill the mesocosms at the start of the experiment (“t0”) (Cornejo
142 et al., 2020; López-Rojo et al., 2022).

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

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

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

175



176

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

183

2.2 Greenhouse gas fluxes

184

185 We measured concentration the of CO_2 and CH_4 (ppm) in the headspace of the
 186 mesocosms during the reference period prior to water addition (“dry”) and immediately after
 187 wetting (“ t_0 ”) both on the first day of the experiment. We subsequently measured after 24 hours,
 188 48 hours, 4 days, 8 days, and 16 days after the water addition. The experiment incubation time
 189 was informed by reported average isolated pool duration (21 days) in the Albarine river in the
 190 summer (Datry, 2017). A PVC foam-bottom lid was securely sealed on top of the mesocosm for
 191 GHG measurement. The lid was equipped with a vent tube (15 cm long, 1 cm diameter) to
 192 equalize the pressure between the mesocosm and the room for ~10 seconds before being closed
 193 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

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

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

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

205 2.3 Statistical analyses

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

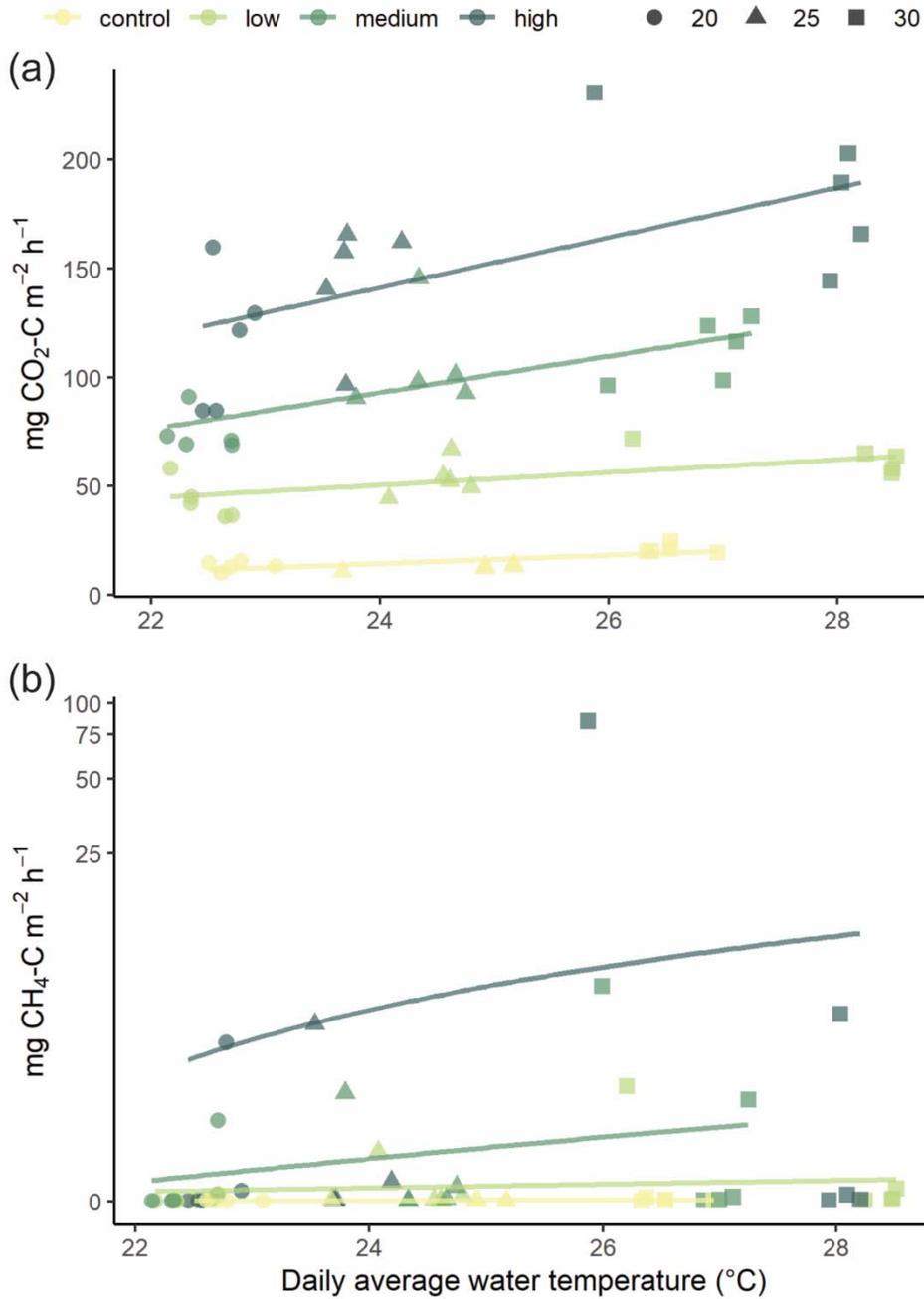
222 3 Results

223 3.1 Effects of temperature and organic matter quantity on greenhouse gas fluxes

224 We observed CO₂ efflux from all of the mesocosms, and CH₄ efflux on all but nine
225 occasions, where we observed weak CH₄ uptake (once during the dry period, and eight times at
226 t0). The average flux during the dry, reference period was 39.22 ± 14.02 mg CO₂-C m⁻² h⁻¹ and
227 0.024 ± 0.014 mg CH₄-C m⁻² h⁻¹. The average flux across all treatments and across the entire
228 experiment period (excluding dry and t0 fluxes) was 78.30 ± 55.06 mg CO₂-C m⁻² h⁻¹ and $1.98 \pm$
229 4.53 mg CH₄-C m⁻² h⁻¹ (Table S2). The highest average flux rate occurred in the 30°C high OM
230 treatments for both CO₂ (189.84 ± 54.00 mg CO₂-C m⁻² h⁻¹) and CH₄ (18.13 ± 44.13 mg CH₄-C
231 m⁻² h⁻¹; Table S2).

232 The most parsimonious model explaining CO₂ fluxes included water temperature and
233 OM quantity (and their interaction). The highest temperature treatment (30°C) had a positive
234 effect on CO₂ fluxes (LMM; $\beta = 0.51$, $p = <0.001$; Table 1). Organic matter had a positive effect
235 on CO₂ fluxes at all treatment levels compared to the control (Table 1). The addition of OM (all
236 treatments averaged) resulted in a CO₂ flux on average 5 times higher than the control (Figure
237 S2). We did not find an interactive effect of temperature and OM quantity on CO₂ fluxes (Table
238 1, Figure 3a).

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



247

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

252

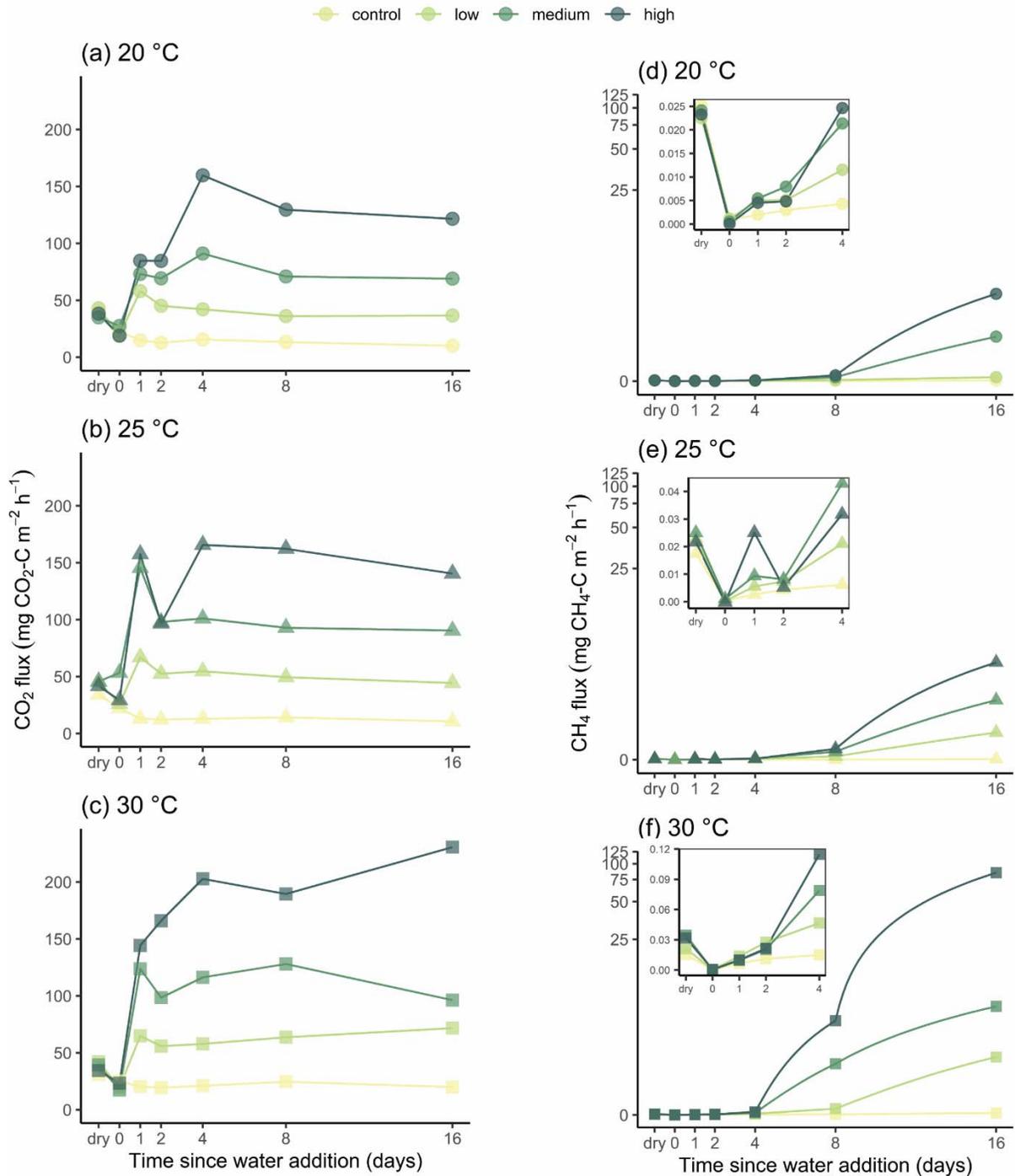
253

254 3.2 Temporal dynamics of greenhouse gas fluxes

255 Date was included in the most parsimonious model for CH₄ fluxes, but not for CO₂ fluxes
256 (Table 1, Table S3). For CH₄, we did not find individual effects of the sampling dates, but rather
257 interactive effects between date and the other treatments. Firstly, the interaction of day 16 and
258 OM (medium and high) had a positive effect on CH₄ fluxes (Table 1). Secondly, day 8 and 16
259 had a positive interactive effect with OM and temperature on CH₄ fluxes (Table 2). If we
260 consider the dry reference period and t0 (not included in our LMMs), we can see a general
261 pattern of a peak in CO₂ 24h after water was added, a small decline to 2 days, before
262 stabilization. The lowest average flux was measured at t0, immediately after water was added
263 (25.81 ± 11.45 mg CO₂-C m⁻² h⁻¹), and the highest average flux was measured on the fourth day
264 of the experiment (88.24 ± 65.02 mg CO₂-C m⁻² h; Figure 4a-c). For CH₄, the lowest average
265 flux was also at t0 (0.48 ± 0.58 μg CH₄-C m⁻² h⁻¹), and the highest was recorded on the 16th day
266 of the experiment ($9240.86 \pm 29,498.55$ μg CH₄-C m⁻² h⁻¹; Figure 4d-f). We were unable to fully
267 investigate the temporal dynamics of DO concentrations, as our first measurement was at 3 days
268 after water was added to the mesocosms (Figure S4). However, we detected hypoxic conditions,
269 approaching anoxic conditions (DO < 0.27 mg L⁻¹) in all of the OM addition treatments since
270 day 3 for the duration of the experiment (Figure S4). Here, we defined hypoxia as DO
271 concentrations <2 mg L⁻¹ and anoxia as the complete absence of oxygen (Pardo & García, 2016).
272 With our available data, we found negative effect of OM (all treatment levels) on DO, and a
273 significant decline in DO on the last day compared to the third day (Figure S4, Table S4).

274 3.3 Effects of temperature and organic matter quantity on leaf litter mass loss

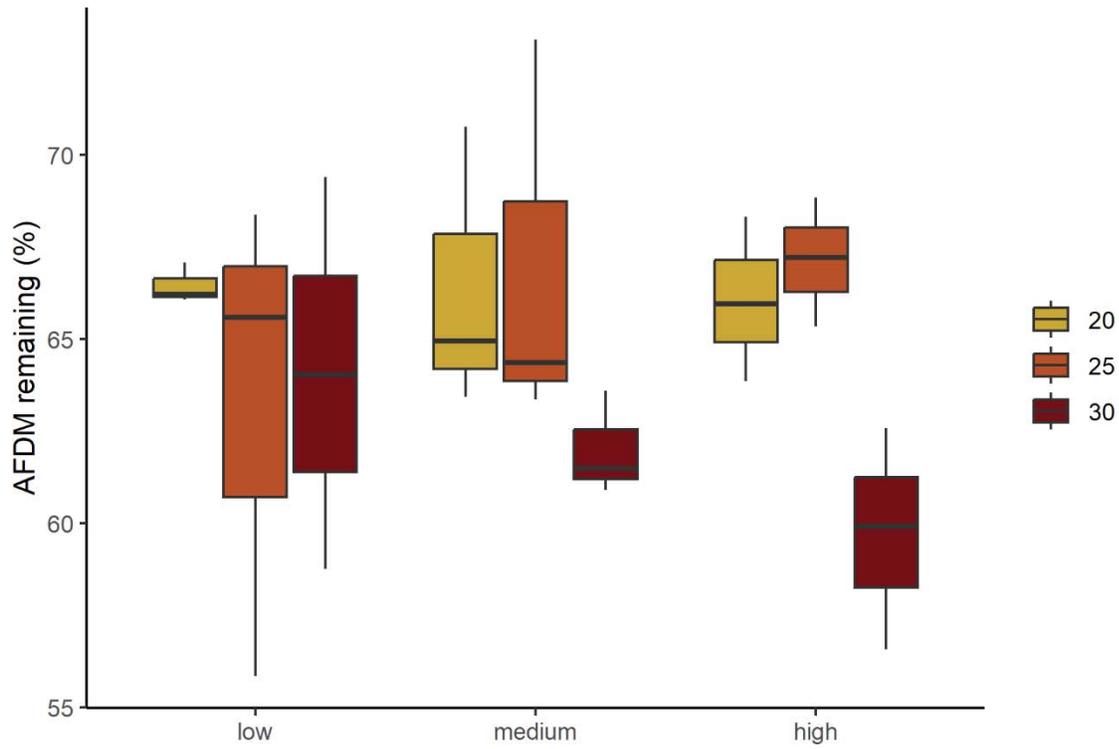
275 We did not find an effect of temperature or OM quantity individually, nor in interaction
276 on the leaf litter AFDM remaining (Table 2; Figure 5). However, we observe a trend in
277 decreasing AFDM remaining at the 30°C temperature treatment with increasing OM (Figure 5).
278 An approximate mass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



281

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.

282



283

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

288

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		

292 4 Discussion

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

318 4.1 Effects of temperature and organic matter quantity on greenhouse gas fluxes

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

322 methanogenesis is consistently reported in the literature from the microbial to the ecosystem
323 scale (Yvon-Durocher et al., 2014). Methanogenesis was found to be disproportionately more
324 sensitive to long-term experimental warming than methanotrophy in artificial ponds (Zhu et al.,
325 2020). Moreover, the temperature dependence of CH₄ emission is estimated to be considerably
326 higher than that of CO₂ (Yvon-Durocher et al., 2014). We did not observe a similar synergistic
327 effect on CO₂ fluxes, but rather positive individual effects of temperature and OM content. In a
328 similar study, CO₂ fluxes from lab incubated streambed sediments increased significantly with
329 increasing temperature (Comer-Warner et al., 2018). An interactive effect of temperature with
330 sediment substrate type (texture and OM content) was detected, with higher CO₂ flux from finer,
331 higher OM content sediments than medium and coarse, lower OM content sediments (Comer-
332 Warner et al., 2018). Their study aimed to simulate surface water with a 5h incubation, whereas
333 in our study we measured over 16 days, simulating realistic isolated pool conditions (Datry,
334 2017). Thus, the lack of synergistic effect on CO₂ here may be related to the rapid depletion of
335 oxygen in the high temperature and high OM mesocosms, resulting in relatively higher
336 production of CH₄ than CO₂.

337 The significant contribution of leaf litter decomposition to the total carbon efflux from
338 isolated pools is highlighted by the control treatment with no OM which consistently had the
339 lowest average CO₂ and CH₄ flux. In fact, the addition of OM (low to high treatments combined)
340 to the sediments resulted in a 5x and 182x increase in CO₂ and CH₄ fluxes, respectively,
341 compared to the control with sediments alone. These results are in contrast with studies of
342 respiration pulses from rewetting of non-perennial rivers. In a global research effort, respiration
343 rates from rewetted sediments were higher, 417 mg CO₂-C m⁻² h⁻¹ (von Schiller et al., 2019) than
344 the rates from rewetted leaf litter alone, 37 mg CO₂-C m⁻² h⁻¹ (Datry et al., 2018), from the same
345 sites. One possible reason for this discrepancy could be that we measured carbon fluxes from
346 sediments and OM together, wherein the sediments may have served as an inoculum of bacteria
347 and fungi for the processing of the OM (Cornut et al., 2010). It may also be explained by the
348 disparate dynamics between sediment rewetting which is a short-term phenomenon, while in
349 isolated pools, there is more time for microbial activity to resume. In addition, in the Datry et al.
350 (2018) study, the leaves were collected from dry streambeds, and therefore may have been
351 subject to preconditioning effects of rain and solar radiation, which reduced their quality and
352 biodegradability when compared to the leaves in our experiment (del Campo et al., 2019). As

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

355 4.2 Temporal dynamics of greenhouse gas fluxes

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

375 The absence of a significant temporal trend in CO₂ fluxes may have been because some
376 of the CH₄ produced was converted to CO₂ through methanotrophy, potentially in aerobic
377 microsites, as has been observed in soils (Khalil & Baggs, 2005; Levy-Booth et al., 2019) and
378 streambed sediments (Shelley et al., 2015). Additionally, in the absence of oxygen, CO₂ can be
379 produced by various fermentation pathways (Fairbairn et al., 2023). The lack of a decrease in
380 CO₂ over time may also be related to the OM availability which was not limiting, as we had >
381 50% AFDM remaining in all treatments at the end of the experiment. In a field study of isolated
382 pools along a non-perennial river, CO₂ production in isolated pools significantly increased with

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

391 **5 Conclusions**

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

407 The dry phases of non-perennial inland waters are not considered in current estimates of
408 global GHG fluxes from freshwaters (Drake et al., 2018; Marcé et al., 2019; Raymond et al.,
409 2013). Here, we show that isolated pools of non-perennial rivers, simulated under controlled
410 laboratory conditions, have the potential to contribute significant CO₂ and CH₄ emissions to the
411 atmosphere under augmented temperature and OM. Given these findings, in addition to what has
412 been reported during sediment drying (Keller et al., 2020; Paranaíba et al., 2021) and rewetting

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

415 **Acknowledgments**

416 This work was supported by INRAE (Institut national de recherche pour l’agriculture,
417 l’alimentation et l’environnement) and EDF (Électricité de France) through the HYNES
418 collaborative framework. We would like to thank Romain Sarremejane who provided an
419 insightful and constructive review of this manuscript.

420 **Author Contributions**

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

424 **Open Research**

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

427

428 **References**

429 Allen, G. H., & Pavelsky, T. M. (2018). Global extent of rivers and streams. *Science*, *361*(6402),
430 585–588.

431 Arora-Williams, K., Holder, C., Secor, M., Ellis, H., Xia, M., Gnanadesikan, A., & Preheim, S.
432 P. (2022). Abundant and persistent sulfur-oxidizing microbial populations are responsive to
433 hypoxia in the Chesapeake Bay. *Environmental Microbiology*, *24*(5), 2315–2332.

434 Bartoń, K. (2019). *MuMIn: Multi-model inference (R Package Version 1.43.6, 2019)*.

435 <https://cran.r-project.org/package=MuMIn>

436 Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models
437 Using lme. *Journal of Statistical Software*, *67*(1), 1–48.

438 <https://doi.org/doi:10.18637/jss.v067.i01>.

- 439 Battin, T. J., Luyssaert, S., Kaplan, L. A., Aufdenkampe, A. K., Richter, A., & Tranvik, L. J.
440 (2009). The boundless carbon cycle. *Nature Geoscience*, 2(9), 598–600.
- 441 Bonada, N., Cañedo-Argüelles, M., Gallart, F., von Schiller, D., Fortuño, P., Latron, J., Llorens,
442 P., Múrria, C., Soria, M., Vinyoles, D., & Cid, N. (2020). Conservation and Management of
443 Isolated Pools in Temporary Rivers. *Water*, 12(10), 2870. <https://doi.org/10.3390/w12102870>
- 444 Boulton, A. J., & Lake, P. S. (1992). Benthic organic matter and detritivorous
445 macroinvertebrates in two intermittent streams in south-eastern Australia. *Hydrobiologia*, 241,
446 107–118.
- 447 Chan, M. A., Moser, K., Davis, J. M., Southam, G., Hughes, K., & Graham, T. (2005). Desert
448 Potholes: Ephemeral Aquatic Microsystems. *Aquatic Geochemistry*, 11(3), 279–302.
449 <https://doi.org/10.1007/s10498-004-6274-8>
- 450 Cole, J. (2013). Freshwater ecosystems and the carbon cycle. *Excellence in Ecology*, 18, 1–142.
- 451 Comer-Warner, S. A., Romeijn, P., Gooddy, D. C., Ullah, S., Kettridge, N., Marchant, B.,
452 Hannah, D. M., & Krause, S. (2018). Thermal sensitivity of CO₂ and CH₄ emissions varies with
453 streambed sediment properties. *Nature Communications*, 9(1), 2803.
454 <https://doi.org/10.1038/s41467-018-04756-x>
- 455 Cornejo, A., Pérez, J., Alonso, A., López-Rojo, N., Monroy, S., & Boyero, L. (2020). A common
456 fungicide impairs stream ecosystem functioning through effects on aquatic hyphomycetes and
457 detritivorous caddisflies. *Journal of Environmental Management*, 263, 110425.
- 458 Cornut, J., Elger, A., Lambrigt, D., Marmonier, P., & Chauvet, E. (2010). Early stages of leaf
459 decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams.
460 *Freshwater Biology*, 55(12), 2541–2556.

- 461 Datry, T. (2017). Ecological Effects of Flow Intermittence in Gravel-Bed Rivers. In *Gravel-Bed*
462 *Rivers* (pp. 261–297). John Wiley & Sons, Ltd. <https://doi.org/10.1002/9781118971437.ch10>
- 463 Datry, T., Foulquier, A., Corti, R., von Schiller, D., Tockner, K., Mendoza-Lera, C., Clement, J.
464 C., Gessner, M. O., Moleon, M., Stubbington, R., Gucker, B., Albarino, R., Allen, D. C.,
465 Altermatt, F., Arce, M., Arnon, S., Banas, D., Banegas-Medina, A., Beller, E., ... Zoppini, A.
466 (2018). A global analysis of terrestrial plant litter dynamics in non-perennial waterways. *Nature*
467 *Geoscience*, *11*(7), 497–503. <https://doi.org/10.1038/s41561-018-0134-4>
- 468 Datry, T., Larned, S. T., & Tockner, K. (2014). Intermittent Rivers: A Challenge for Freshwater
469 Ecology. *Bioscience*, *64*(3), 229–235. <https://doi.org/10.1093/biosci/bit027>
- 470 Datry, T., Truchy, A., Olden, J. D., Busch, M. H., Stubbington, R., Dodds, W. K., Zipper, S., Yu,
471 S., Messenger, M. L., Tonkin, J. D., Kaiser, K. E., Hammond, J. C., Moody, E. K., Burrows, R.
472 M., Sarremejane, R., DelVecchia, A. G., Fork, M. L., Little, C. J., Walker, R. H., ... Allen, D.
473 (2023). Causes, Responses, and Implications of Anthropogenic versus Natural Flow
474 Intermittence in River Networks. *BioScience*, *73*(1), 9–22. <https://doi.org/10.1093/biosci/biac098>
- 475 Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition
476 and feedbacks to climate change. *Nature*, *440*(7081), 165–173.
- 477 Day, J. A., Malan, H. L., Malijani, E., & Abegunde, A. P. (2019). Water quality in non-perennial
478 rivers. *Water SA*, *45*(3), 487–500.
- 479 del Campo, R., Gómez, R., & Singer, G. (2019). Dry phase conditions prime wet-phase
480 dissolved organic matter dynamics in intermittent rivers. *Limnology and Oceanography*, *64*(5),
481 1966–1979.

- 482 Döll, P., & Schmied, H. M. (2012). How is the impact of climate change on river flow regimes
483 related to the impact on mean annual runoff? A global-scale analysis. *Environmental Research*
484 *Letters*, 7(1), 014037.
- 485 Drake, T. W., Raymond, P. A., & Spencer, R. G. M. (2018). Terrestrial carbon inputs to inland
486 waters: A current synthesis of estimates and uncertainty. *Limnology and Oceanography Letters*,
487 3(3), 132–142. <https://doi.org/10.1002/lol2.10055>
- 488 Fairbairn, L., Rezanezhad, F., Gharasoo, M., Parsons, C. T., Macrae, M. L., Slowinski, S., &
489 Van Cappellen, P. (2023). Relationship between soil CO₂ fluxes and soil moisture: Anaerobic
490 sources explain fluxes at high water content. *Geoderma*, 434, 116493.
491 <https://doi.org/10.1016/j.geoderma.2023.116493>
- 492 Fellman, J. B., Dogramaci, S., Skrzypek, G., Dodson, W., & Grierson, P. F. (2011). Hydrologic
493 control of dissolved organic matter biogeochemistry in pools of a subtropical dryland river.
494 *Water Resources Research*, 47(6).
- 495 Gómez-Gener, L., Lupon, A., Laudon, H., & Sponseller, R. A. (2020). Drought alters the
496 biogeochemistry of boreal stream networks. *Nature Communications*, 11(1), Article 1.
497 <https://doi.org/10.1038/s41467-020-15496-2>
- 498 Gómez-Gener, L., Obrador, B., von Schiller, D., Marcé, R., Casas-Ruiz, J. P., Proia, L., Acuña,
499 V., Catalán, N., Muñoz, I., & Koschorreck, M. (2015). Hot spots for carbon emissions from
500 Mediterranean fluvial networks during summer drought. *Biogeochemistry*, 125(3), 409–426.
501 <https://doi.org/10.1007/s10533-015-0139-7>
- 502 Gómez-Gener, L., Siebers, A. R., Arce, M. I., Arnon, S., Bernal, S., Bolpagni, R., Datry, T.,
503 Gionchetta, G., Grossart, H.-P., Mendoza-Lera, C., Pohl, V., Risse-Buhl, U., Shumilova, O.,
504 Tzoraki, O., von Schiller, D., Weigand, A., Weigelhofer, G., Zak, D., & Zoppini, A. (2021).

505 Towards an improved understanding of biogeochemical processes across surface-groundwater
506 interactions in intermittent rivers and ephemeral streams. *Earth-Science Reviews*, 220, 103724.
507 <https://doi.org/10.1016/j.earscirev.2021.103724>

508 Granados, V., Gutiérrez-Cánovas, C., Arias-Real, R., Obrador, B., Harjung, A., & Butturini, A.
509 (2020). The interruption of longitudinal hydrological connectivity causes delayed responses in
510 dissolved organic matter. *Science of the Total Environment*, 713, 136619.

511 Hutchinson, G. L., & Livingston, G. P. (2001). Vents and seals in non-steady-state chambers
512 used for measuring gas exchange between soil and the atmosphere. *European Journal of Soil
513 Science*, 52(4), 675–682.

514 Jocque, M., Vanschoenwinkel, B., & Brendonck, L. (2010). Freshwater rock pools: A review of
515 habitat characteristics, faunal diversity and conservation value. *Freshwater Biology*, 55(8),
516 1587–1602. <https://doi.org/10.1111/j.1365-2427.2010.02402.x>

517 Keller, P. S., Catalán, N., von Schiller, D., Grossart, H.-P., Koschorreck, M., Obrador, B., Frassl,
518 M. A., Karakaya, N., Barros, N., Howitt, J. A., Mendoza-Lera, C., Pastor, A., Flaim, G., Aben,
519 R., Riis, T., Arce, M., Onandia, G., Paranaíba, J. R., Linkhorst, A., ... Marcé, R. (2020). Global
520 CO₂ emissions from dry inland waters share common drivers across ecosystems. *Nature
521 Communications*, 11(1), 2126. <https://doi.org/10.1038/s41467-020-15929-y>

522 Khalil, M. I., & Baggs, E. M. (2005). CH₄ oxidation and N₂O emissions at varied soil water-
523 filled pore spaces and headspace CH₄ concentrations. *Soil Biology and Biochemistry*, 37(10),
524 1785–1794.

525 Levy-Booth, D. J., Giesbrecht, I. J. W., Kellogg, C. T. E., Heger, T. J., D'Amore, D. V., Keeling,
526 P. J., Hallam, S. J., & Mohn, W. W. (2019). Seasonal and ecohydrological regulation of active

527 microbial populations involved in DOC, CO₂, and CH₄ fluxes in temperate rainforest soil. *The*
528 *ISME Journal*, 13(4), Article 4. <https://doi.org/10.1038/s41396-018-0334-3>

529 López-Rojo, N., Boyero, L., Pérez, J., Basaguren, A., & Cardinale, B. J. (2022). No evidence of
530 biodiversity effects on stream ecosystem functioning across green and brown food web
531 pathways. *Freshwater Biology*, 67(4), 720–730.

532 López-Rojo, N., Datry, T., Peñas, F. J., Singer, G., Lamouroux, N., Barquín, J., Rodeles, A. A.,
533 Silverthorn, T., Sarremejane, R., del Campo, R., Estévez, E., Mimeau, L., Boyer, F., Annika, &
534 Foulquier, A. (2023). Carbon emissions from rivers may be underestimated: Evidence from
535 European drying networks. *Nature Geoscience*, Preprint.
536 <https://www.researchsquare.com/article/rs-3006027/v1>

537 Lü, W., Ren, H., Ding, W., Li, H., Yao, X., & Jiang, X. (2023). The effects of climate warming
538 on microbe-mediated mechanisms of sediment carbon emission. *Journal of Environmental*
539 *Sciences*, 129, 16–29. <https://doi.org/10.1016/j.jes.2022.09.016>

540 Luo, Y., & Zhou, X. (2010). *Soil respiration and the environment*. Elsevier.

541 Marcé, R., Obrador, B., Gómez-Gener, L., Catalán, N., Koschorreck, M., Arce, M. I., Singer, G.,
542 & von Schiller, D. (2019). Emissions from dry inland waters are a blind spot in the global carbon
543 cycle. *Earth-Science Reviews*, 188, 240–248. <https://doi.org/10.1016/j.earscirev.2018.11.012>

544 Maswanganye, S. E., Dube, T., Mazvimavi, D., & Jovanovic, N. (2022). Remotely sensed
545 applications in monitoring the spatio-temporal dynamics of pools and flows along non-perennial
546 rivers: A review. *South African Geographical Journal*, 104(4), 427–445.

547 Messenger, M. L., Lehner, B., Cockburn, C., Lamouroux, N., Pella, H., Snelder, T., Tockner, K.,
548 Trautmann, T., Watt, C., & Datry, T. (2021). Global prevalence of non-perennial rivers and
549 streams. *Nature*, 594(7863), 391–397.

550 Michel, A., Schaeffli, B., Wever, N., Zekollari, H., Lehning, M., & Huwald, H. (2021). Future
551 water temperature of rivers in Switzerland under climate change investigated with physics-based
552 models. *Hydrology and Earth System Sciences Discussions*.

553 Myhre, G., Shindell, D., Bréon, F.-M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D.,
554 Lamarque, J. F., & Lee, D. (2013). *Anthropogenic and Natural Radiative Forcing*. In: *Climate*
555 *Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth*
556 *Assessment Report of the Intergovernmental Panel on Climate Change*.

557 Paranaíba, J. R., Aben, R., Barros, N., Quadra, G., Linkhorst, A., Amado, A. M., Brothers, S.,
558 Catalán, N., Condon, J., Finlayson, C. M., Grossart, H.-P., Howitt, J., Oliveira Junior, E. S.,
559 Keller, P. S., Koschorreck, M., Laas, A., Leigh, C., Marcé, R., Mendonça, R., ... Kosten, S.
560 (2021). Cross-continental importance of CH₄ emissions from dry inland-waters. *Science of the*
561 *Total Environment*, 814, 1–11. <https://doi.org/10.1016/j.scitotenv.2021.151925>

562 Pardo, I., & García, L. (2016). Water abstraction in small lowland streams: Unforeseen hypoxia
563 and anoxia effects. *Science of The Total Environment*, 568, 226–235.
564 <https://doi.org/10.1016/j.scitotenv.2016.05.218>

565 R Core Team. (2022). *R: A language and environment for statistical computing*. [https://www.R-](https://www.R-project.org/)
566 [project.org/](https://www.R-project.org/).

567 Raymond, P. A., Hartmann, J., Lauerwald, R., Sobek, S., McDonald, C., Hoover, M., Butman,
568 D., Striegl, R., Mayorga, E., Humborg, C., Kortelainen, P., Dürr, H., Meybeck, M., Ciais, P., &
569 Guth, P. (2013). Global carbon dioxide emissions from inland waters. *Nature*, 503(7476), Article
570 7476. <https://doi.org/10.1038/nature12760>

- 571 Regnier, P., Resplandy, L., Najjar, R. G., & Ciais, P. (2022). The land-to-ocean loops of the
572 global carbon cycle. *Nature*, *603*(7901), Article 7901. [https://doi.org/10.1038/s41586-021-](https://doi.org/10.1038/s41586-021-04339-9)
573 04339-9
- 574 Reverey, F., Grossart, H.-P., Premke, K., & Lischeid, G. (2016). Carbon and nutrient cycling in
575 kettle hole sediments depending on hydrological dynamics: A review. *Hydrobiologia*, *775*(1), 1–
576 20. <https://doi.org/10.1007/s10750-016-2715-9>
- 577 Romeijn, P., Comer-Warner, S. A., Ullah, S., Hannah, D. M., & Krause, S. (2019). Streambed
578 organic matter controls on carbon dioxide and methane emissions from streams. *Environmental*
579 *Science & Technology*, *53*(5), 2364–2374. <https://doi.org/10.1021/acs.est.8b04243>
- 580 Schreckinger, J., Mutz, M., Mendoza-Lera, C., & Frossard, A. (2021). Attributes of drying
581 define the structure and functioning of microbial communities in temperate riverbed sediment.
582 *Frontiers in Microbiology*, *12*, 676615.
- 583 Serrano-Silva, N., Sarria-Guzmán, Y., Dendooven, L., & Luna-Guido, M. (2014).
584 Methanogenesis and methanotrophy in soil: A review. *Pedosphere*, *24*(3), 291–307.
585 [https://doi.org/10.1016/S1002-0160\(14\)60016-3](https://doi.org/10.1016/S1002-0160(14)60016-3)
- 586 Shelley, F., Abdullahi, F., Grey, J., & Trimmer, M. (2015). Microbial methane cycling in the bed
587 of a chalk river: Oxidation has the potential to match methanogenesis enhanced by warming.
588 *Freshwater Biology*, *60*(1), 150–160.
- 589 Siebers, A. R., Pettit, N. E., Skrzypek, G., Dogramaci, S., & Grierson, P. F. (2020). Diel cycles
590 of $\delta^{13}\text{C}_{\text{DIC}}$ and ecosystem metabolism in ephemeral dryland streams. *Aquatic Sciences*, *82*(2),
591 32. <https://doi.org/10.1007/s00027-020-0708-2>

- 592 van Vliet, M. T. H., Franssen, W. H. P., Yearsley, J. R., Ludwig, F., Haddeland, I., Lettenmaier,
593 D. P., & Kabat, P. (2013). Global river discharge and water temperature under climate change.
594 *Global Environmental Change*, 23(2), 450–464. <https://doi.org/10.1016/j.gloenvcha.2012.11.002>
- 595 Vazquez, E., Amalfitano, S., Fazi, S., & Butturini, A. (2011). Dissolved organic matter
596 composition in a fragmented Mediterranean fluvial system under severe drought conditions.
597 *Biogeochemistry*, 102(1), 59–72. <https://doi.org/10.1007/s10533-010-9421-x>
- 598 von Schiller, D., Datry, T., Corti, R., Foulquier, A., Tockner, K., Marcé, R., García-Baquero, G.,
599 Odriozola, I., Obrador, B., Elozegi, A., Mendoza-Lera, C., Gessner, M. O., Stubbington, R.,
600 Albariño, R., Allen, D. C., Altermatt, F., Arce, M. I., Arnon, S., Banas, D., ... Zoppini, A.
601 (2019). Sediment respiration pulses in intermittent rivers and ephemeral streams. *Global*
602 *Biogeochemical Cycles*, 33(10), 1251–1263. <https://doi.org/10.1029/2019GB006276>
- 603 von Schiller, D., Marcé, R., Obrador, B., Gómez-Gener, L., Casas-Ruiz, J. P., Acuña, V., &
604 Koschorreck, M. (2014). Carbon dioxide emissions from dry watercourses. *Inland Waters*, 4(4),
605 377–382. <https://doi.org/10.5268/IW-4.4.746>
- 606 Watanabe, T., Kimura, M., & Asakawa, S. (2007). Dynamics of methanogenic archaeal
607 communities based on rRNA analysis and their relation to methanogenic activity in Japanese
608 paddy field soils. *Soil Biology and Biochemistry*, 39(11), 2877–2887.
- 609 Yu, S., Burrows, R. M., Shanafield, M., & Kennard, M. J. (2022). Water-level recession
610 characteristics in isolated pools within non-perennial streams. *Advances in Water Resources*,
611 166, 104267. <https://doi.org/10.1016/j.advwatres.2022.104267>
- 612 Yvon-Durocher, G., Allen, A. P., Bastviken, D., Conrad, R., Gudas, C., St-Pierre, A., Thanh-
613 Duc, N., & Del Giorgio, P. A. (2014). Methane fluxes show consistent temperature dependence
614 across microbial to ecosystem scales. *Nature*, 507(7493), 488–491.

615 Zhu, Y., Purdy, K. J., Eyice, Ö., Shen, L., Harpenslager, S. F., Yvon-Durocher, G., Dumbrell, A.
616 J., & Trimmer, M. (2020). Disproportionate increase in freshwater methane emissions induced
617 by experimental warming. *Nature Climate Change*, *10*(7), 685–690.

618 Zimmer, M. A., Kaiser, K. E., Blaszcak, J. R., Zipper, S. C., Hammond, J. C., Fritz, K. M.,
619 Costigan, K. H., Hosen, J., Godsey, S. E., & Allen, G. H. (2020). Zero or not? Causes and
620 consequences of zero-flow stream gage readings. *Wiley Interdisciplinary Reviews: Water*, *7*(3),
621 e1436.

622

623

624

625

626

627

628