

# Drying, more than warming, alters ecosystem functioning in streams with different energy pathways

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## Abstract

1. Empirical evidence and theory suggest that climate warming and an increase in the frequency and duration of drying events will alter the metabolic balance of freshwater ecosystems. However, the impacts of climate change on ecosystem metabolism may depend on whether energy inputs are of autochthonous or allochthonous origin. To date, few studies have examined how warming and drying may interact to alter stream metabolism, much less how their impacts may depend on the energy-base of the food web.
2. To address this research gap, we conducted a multi-factorial experiment using outdoor mesocosms to investigate the individual and synergistic effects of warming and drought on metabolic processes in stream mesocosms with green (algal-based) vs. mixed (algal- and detritus-based) vs. brown (detritus-based) energy pathways.
3. We set up 48 mesocosms with one of three different levels of shade and leaf litter input combinations to create mesocosms with different primary energy channels. In addition, we warmed half of the mesocosms by ~2–3°C. We assessed changes in ecosystem respiration (ER), gross primary production (GPP), net ecosystem production (NEP) and organic matter biomass in warmed and ambient temperature mesocosms before a 24 day drying event and after rewetting.
4. Surprisingly, experimental warming had little effect on metabolic processes. Drying, however, led to decreased rates of ER and GPP and led to an overall reduction in NEP. Although the effects of drying were similar across energy channel treatments, reductions in ER and GPP were primarily driven by decreases in biomass of benthic and filamentous algae.
5. Overall, we demonstrate that drying led to lower rates of NEP in mesocosms regardless of energy inputs. While warming showed little effect in our study, our results suggest that an increase in the frequency of stream drying events could greatly alter the metabolic balance of many aquatic ecosystems.

## KEYWORDS

carbon cycle, climate change, ecosystem metabolism, energy channels, mesocosm, stream drying, warming

## 1 | INTRODUCTION

Despite covering less than 4% of Earth's surface, inland freshwaters play a disproportionately large role in the global carbon cycle through their high rates of carbon sequestration and respiration (Cole et al., 2007; Downing, 2010; Tranvik et al., 2009; Verpoorter et al., 2014). Recent estimates suggest that 0.75–2.1 petagrams (Pg) of carbon are emitted from inland freshwaters each year (Cole et al., 2007; Raymond et al., 2013). Additionally, as much as 5.1 Pg of terrestrial fixed carbon is transferred from terrestrial ecosystems to inland freshwaters annually (Drake et al., 2018), making inland waters a major pool of carbon and globally relevant to the carbon cycle. However, recent evidence has highlighted the potential for alterations to the biogeochemical cycling of carbon in freshwaters due to climate change (Quan et al., 2019; Schroter et al., 2005; Yvon-Durocher et al., 2010). Given the importance of inland freshwaters to the global carbon cycle, an improved understanding of how climate change will alter the sequestration and respiration of carbon is crucial for the future management of freshwaters and terrestrial ecosystems worldwide.

Freshwater metabolism, or the balance between the processes of gross primary production (GPP) and ecosystem respiration (ER), is fundamental to the freshwater carbon cycle (Hotchkiss et al., 2015). Because the processes of GPP and ER are both strongly temperature dependent (Allen et al., 2005; López-Urrutia et al., 2006; Yvon-Durocher et al., 2010), climate warming is predicted to increase rates of carbon fixation and emissions from freshwaters. However, evidence suggests that the activation energies ( $E_a$ ) of photosynthesis and ecosystem respiration may be different (photosynthesis  $E_a \approx 0.32$  eV; Allen et al., 2005; López-Urrutia et al., 2006; respiration  $E_a \approx 0.60$ – $0.70$  eV; Enquist et al., 2003; Gillooly et al., 2001; but see Demars et al., 2016). Accordingly, environmental warming should alter the metabolic balance of freshwater ecosystems, as carbon remineralization by respiration should increase more rapidly than carbon fixation by photosynthesis (Yvon-Durocher et al., 2010). Therefore, net ecosystem production (NEP) is predicted to decrease in many freshwater ecosystems as the Earth warms, potentially making these systems more heterotrophic (Song et al., 2018; Yvon-Durocher et al., 2010, 2017).

In addition to warmer temperatures, climate change is intensifying the frequency, duration and magnitude of drying events in streams and rivers worldwide (IPCC, 2014; Zipper et al., 2021). However, the effects of drying on stream ecosystem metabolism are not well understood. There are a limited number of studies that have described changes in ecosystem metabolism during periods of reduced flow, water absence and upon return of flow in streams (but see Acuña et al., 2004, 2015; Zlatanović et al., 2018). Reduced flow and non-flow (i.e. a lack of surface water in streams) events alter the structure and physiological responses of both autotrophic and heterotrophic components of epilithic biofilms, which can result in significant changes to ecosystem metabolism (Sabater et al., 2016). For example, streambed desiccation can reduce both autotrophic and heterotrophic biomass and thus reduce rates of GPP and ER

(Timoner et al., 2012). Among the reported effects of drought on ecosystem metabolism, perhaps the most notable is the differential effect of exposure to non-flow events on autotrophs and heterotrophs (Acuña et al., 2015; Sabater et al., 2016). Non-flow events have stronger negative effects on autotrophs when compared to heterotrophic components of biofilms (Acuña et al., 2015; Timoner et al., 2012). Thus, like warming, non-flow events are hypothesized to result in lower rates of NEP in many freshwater ecosystems as autotrophic processes are less resistant to drying (Acuña et al., 2015).

While empirical evidence and theory both suggest that warming temperatures and an increased frequency of drying events can alter stream metabolism, their impacts may depend on whether metabolic processes are driven primarily by autochthonous or allochthonous energy inputs. For example, open-canopy streams receive large amounts of sunlight, which fuels algal production and drives high rates of GPP, thereby creating ecosystems fueled primarily by primary producers (i.e. 'green' energy channels). In contrast, forested streams with extensive canopy cover receive large amounts of allochthonous inputs such as leaf litter but little sunlight, which leads to high rates of ER and low rates of GPP (Mulholland et al., 2001). Consequently, ER exceeds GPP in many streams and rivers, resulting in net heterotrophy and detritus-based or 'brown' energy channels (Cole & Caraco, 2001; Marcarelli et al., 2011; Mulholland et al., 2001). Given the dissimilarity in the response to drying between autotrophic and heterotrophic communities and the difference in the temperature dependence between carbon fixation and remineralization, it is likely that the source of carbon (allochthonous vs. autochthonous-derived) can control metabolic processes in streams and could modulate the effects of climate change on ecosystem metabolism.

Despite the documented evidence showing the individual effects of warming and drought on freshwater ecosystem processes (Riis et al., 2017; Yvon-Durocher et al., 2010), few studies have examined how warming and drying may interact to alter stream metabolism (but see Arias Font et al., 2021) and how their impacts may depend on the energy-base of the food web. This knowledge gap limits generalization about the impacts of warming and drought on ecosystems and weakens our ability to predict which ecosystems will be most affected by climate change. In this experiment, we investigated the individual and combined effects of warming and drying on metabolism in stream mesocosms with green (algal dominated) versus mixed (algal- and detritus-based) versus brown (detritus dominated) energy pathways. We tested the following hypotheses: (1) warming will stimulate heterotrophic processes more than autotrophic processes in all energy channels, thereby increasing rates of ER more than GPP; (2) stream drying will negatively impact both ER and GPP, but drying will reduce GPP more than ER in all energy channels; (3) warming and drying will interact synergistically, reducing GPP more than ER and resulting in lower rates of NEP in all energy channels; (4) the synergistic effect of warming and drought on NEP will be greater in mesocosms with green energy channels than those with brown energy channels because of the greater reliance on autotrophic versus allochthonous organic matter in green energy channel

treatments and the greater negative effect of drying on autotrophic communities.

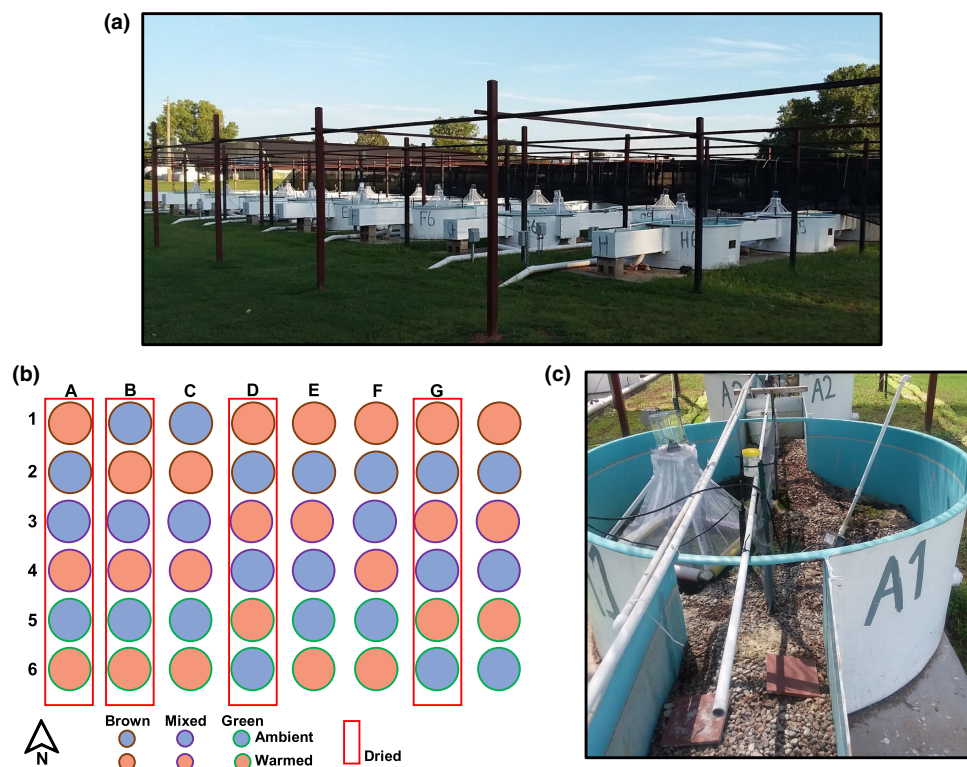
## 2 | MATERIALS AND METHODS

### 2.1 | Experimental set-up

Our study was conducted in 48 outdoor stream mesocosms (Figure 1) located at the University of Oklahoma Aquatic Research Facility in Norman, Oklahoma, USA during April–November 2018 (Table S1). Each mesocosm consisted of a 0.60m<sup>2</sup> rectangular run at the upstream end flowing into a 2.54m<sup>2</sup> circular pool and out through a 0.32m<sup>2</sup> rectangular run at the downstream end as described in Matthews et al. (2006). Mesocosms were scoured with a high-pressure washer and drained to eliminate senescent algae and other debris prior to the start of the experiment. Gravel substrate (diameter ~10–20mm) was distributed evenly throughout the riffles and pools. On 30 April 2018, mesocosms were filled with local groundwater and were seeded with approximately 5L of gravel from a nearby stream on 1 May 2018 to introduce microbial and algal propagules (Table S1). An aquarium pump (Danner Utility Pump Model 7; 2650L/h pump rate) created flow by drawing water from the downstream run and pumping it to the upstream run through a

PVC pipe. Each day, approximately 360L of fresh groundwater were added to each mesocosm and a standpipe maintained a constant water level. Water temperature and light were monitored in each mesocosm using HOBO loggers (Onset HOBO MX2202).

We created stream mesocosms with different primary energy channels (i.e. green vs. mixed vs. brown), by manipulating the amount of ambient sunlight mesocosms received and by adding different amounts of dried leaf litter. Mesocosms with primarily green energy channels (hereafter 'green mesocosms') were left open to direct sunlight and received 35g of dried leaf litter, which consisted of approximately equal parts sycamore *Platanus occidentalis*, eastern cottonwood *Populus deltoides* and northern red oak *Quercus rubra* leaves. These are common tree species found in forested and riparian areas of the southern Great Plains. Mesocosms with a combination of green and brown energy channels (hereafter 'mixed mesocosms') were covered with one layer of shade cloth (80% reduction in ambient sunlight) and received 150g of leaf litter. Streams with primarily brown energy channels (hereafter 'brown mesocosms') were covered with two layers of shade cloth which reduced ambient sunlight by 96% and received 350g of dried leaf litter. We added leaf litter directly to the pools of the mesocosms and allowed the leaf litter to settle to the bottoms of the mesocosms naturally. Initially, we added leaf litter on 18 May 2018 and replenished leaf litter lost to microbial breakdown and



**FIGURE 1** (a) Photograph of stream mesocosms. (b) Diagram of the layout of the experimental treatments in the 48 stream mesocosms. Experimental treatments consisted of warming: ambient ( $n=24$ ) and warmed ( $n=24$ ); drying: not dried ( $n=24$ ) and dried ( $n=24$ ); energy channel: brown ( $n=16$ ), mixed ( $n=16$ ) and green ( $n=16$ ). (c) Photograph of one of the dried mesocosms during the drying phase of the experiment.

invertebrate consumption by adding an additional 20, 100, and 150 g to the green, mixed and brown mesocosms, respectively, on 17 July 2018 and 30 September 2018 (Table S1). Leaf litter was added to reflect inputs of leaf litter to natural streams from different biomes across North America (Benfield, 1997).

Submersible water heaters (Finnex Deluxe TH-0500S) were added to half of the mesocosms within each energy channel treatment on 9 May 2018 (Table S1). Water heaters maintained a continuous 2–3°C increase above ambient temperatures of unheated mesocosms (Table 1), which falls within the projections of increase for North American surface waters over the next century (IPCC, 2013). However, due to differences in shading among energy channel treatments, water temperatures were generally highest in green mesocosms and lowest in brown mesocosms (Table 1). To simulate a stream drying event, half of the mesocosms within each energy channel × warming treatment were dewatered (hereafter 'dry mesocosms' vs. 'wet mesocosms'). We began drying mesocosms slowly on 28 August 2018 (Table S1) and no surface water remained after ~48 h. However, subsurface water within interstitial gravel space was still present. During this drying phase of the experiment, water heaters were turned off in the dry mesocosms. Mesocosms remained without surface water for 24 days and were refilled on 22 September 2018 at a rate of ~1 L/min. Water heaters were turned back on once mesocosms were refilled. See Supporting Information (Appendix S1) for more detail regarding experimental design. This experiment did not require any licenses or permits to be carried out.

## 2.2 | Benthic metabolism measurements

We measured benthic metabolism using light and dark incubations of benthic substrate in portable recirculating chambers (see Rüegg et al., 2015 for full description of chambers). Plastic mesh baskets (10 × 10 × 6 cm, mesh size ≈ 1 cm) were filled with gravel substrate and buried flush with the sediment in the pool section of each mesocosm at least 1 month prior to being used in chamber metabolism measurements. For each measurement, three mesh baskets filled with substrate and any attached filamentous algae or leaves were included in metabolism measurements. We filled each chamber with three mesh baskets and water from their respective mesocosms. All chamber metabolism measurements were conducted outside of the mesocosms in direct sunlight. Light was measured as photosynthetically active radiation (PAR;  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) with an Apogee quantum sensor (Apogee Instruments, Inc.). Chambers were run for approximately 20 min in the dark followed by 20 min in full sunlight. Dark conditions were achieved by covering the chambers with a thick, dark cloth. PAR measurements were always  $0 \mu\text{mol m}^{-2} \text{s}^{-1}$  under the cloth during dark measurements. Temperature (°C) and DO concentrations were measured every 30 s using YSI ProODO meters. On average, water temperature inside the chambers increased 1.05°C during measurements. In most cases, five chambers were run simultaneously.

Rates of metabolism were calculated from the slope of the linear regression of DO concentrations versus time and accounted for specific substrate basket areas and chamber volumes (Grace & Imberger, 2006), resulting in units of  $\text{mg O}_2 \text{m}^{-2} \text{h}^{-1}$ . Net ecosystem production (NEP) was calculated from the light measurements, while respiration (ER) was calculated from the dark measurements. GPP was calculated as the sum of ER and NEP. During all light measurements, PAR was  $>300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; thus, we assumed that rates represented photosaturated conditions (Hill, 1996). Values for GPP and ER given in units of  $\text{mg O}_2 \text{m}^{-2} \text{h}^{-1}$  were converted to units of carbon (C) using a photosynthetic quotient of 1.2 and a respiratory quotient of 0.85 (Bott, 2006).

After metabolism measurements, we processed the substrates from the chambers for filamentous algae and leaf litter biomass, benthic chlorophyll-*a* and benthic ash-free dry mass (AFDM). The contents of the chambers (three mesh baskets, organic matter and water) were placed in a 5-gallon bucket and agitated by vigorously mixing the contents by hand. Subsamples of the resulting suspended material were filtered onto  $0.7 \mu\text{m}$  glass fibre filters (Whatman GF/F). Filters were stored at  $-20^\circ\text{C}$  and later analyzed for chlorophyll-*a* and AFDM. Chlorophyll-*a* was extracted for approximately 24 h in 90% acetone following Steinman et al. (2007) and measured with a Thermo Scientific GENESYS 10S UV-vis spectrophotometer using standard methods (APHA, 1995). AFDM filters were dried at  $55^\circ\text{C}$  for at least 72 h, allowed to cool overnight in a desiccator, and then reweighed. After filters were reweighed, they were combusted at  $500^\circ\text{C}$  for at least 4 h, allowed to cool and reweighed to obtain AFDM. The remainder of the sample from the bucket was preserved in 70% EtOH and later processed for filamentous algae and leaf litter biomass in the laboratory. Briefly, we removed leaf litter and filamentous algae separately by hand from the samples. Leaf litter and algae were dried at  $55^\circ\text{C}$ , allowed to cool overnight in a desiccator, reweighed for dry mass, combusted at  $500^\circ\text{C}$  for at least 4 h, allowed to cool and reweighed to obtain AFDM.

## 2.3 | Data analysis

Treatment effects on ER, NEP, GPP, leaf litter biomass, algal biomass, benthic chlorophyll *a* and benthic AFDM were tested with linear mixed effects models using the *lme* function in the 'nlme' package (Pinheiro et al., 2019) in the R platform (R Core Team, 2019). Models included warming treatment (ambient, warmed), energy channel (brown, mixed, green), drying treatment (not dried, dried), and time period (pre-drying, post-drying) as fixed factors. Of particular importance is the interaction of drying × period, as it can be used to test the hypothesis that mesocosms that were dried diverged in time from those that were not dried. Individual mesocosm nested with sampling date was treated as a random factor. We did not include sampling date as a fixed factor due to the number of dependent variables and sampling dates. This allowed us to interpret the effects of the different treatments that were apparent between the

pre-drying and post-drying periods of the experiment but not those that emerged at specific time points.

To identify the most parsimonious models and assess significance of the fixed factors, we used the multi-model inference approach (Burnham & Anderson, 2004). Briefly, we started with a global model in which the fixed effects component contained all explanatory variables and their interactions. Then, we generated a set of sub-models using the *dredge* function in the 'MuMIn' package (Bartoń, 2019). We used Akaike's information criterion corrected for small sample sizes (AICc) to select independent variables influencing the dependent variables and Akaike weights and  $\Delta$ AICc to inform model selection. A  $\Delta$ AICc <2 was interpreted as substantial support that the model belonged to the set of best models. If more than one model had  $\Delta$ AICc of 2 or less, model averaging was used to

calculate the variable coefficients (Bolker et al., 2009) and evaluate the significance of the fixed effects terms using the 'MuMIn' package (Bartoń, 2019). Final models were refitted using restricted maximum likelihood (REML) to determine parameter estimates. Assumptions of normality and homoscedasticity were checked prior to model construction. All response variables were log-transformed for model construction. A list of the fixed effects structure of the linear mixed effects sub-models is provided in Supporting Information (Table S2).

### 3 | RESULTS

#### 3.1 | Benthic organic matter biomass

Benthic organic matter biomass was measured on seven dates between 21 June and 17 November 2018 (Table S1). Leaf litter biomass ranged from 0.00 to 122.75 g AFDM m<sup>-2</sup> and was significantly different among all energy channels but not warming or drying treatments (Figure 2; Table S3). Leaf litter biomass was significantly higher in the brown (mean  $\pm$  SE: 25.57  $\pm$  2.23 g AFDM m<sup>-2</sup>) versus the mixed (mean  $\pm$  SE: 9.93  $\pm$  1.07 g AFDM m<sup>-2</sup>) versus the green (mean  $\pm$  SE: 2.45  $\pm$  0.34 g AFDM m<sup>-2</sup>) mesocosms (Table S3). Algal biomass ranged from 0.00 to 394.22 g AFDM m<sup>-2</sup> and was significantly higher in the green (mean  $\pm$  SE: 48.26  $\pm$  4.84 g AFDM m<sup>-2</sup>) and mixed (mean  $\pm$  SE: 42.37  $\pm$  4.84 g AFDM m<sup>-2</sup>) mesocosms than in the brown (mean  $\pm$  SE: 12.90  $\pm$  2.85 g AFDM m<sup>-2</sup>) mesocosms (Table S3; Figure S1). Algal biomass was not affected by warming but was significantly reduced by drying (Figure 2; Table S3; Drying  $\times$  Period,  $p$  < 0.001). However, the effect of drying on algal biomass was similar across energy channels (Table S3). Over the course of the experiment, benthic chlorophyll *a* ranged from <0.01 to 0.386 g m<sup>-2</sup>. Chlorophyll *a* was not affected by warming but was significantly

TABLE 1 Summarized daily water temperature data for mesocosms from 5 May 2018 to 17 November 2018.

	Temperature (°C)			
	Not dried		Dried	
	Mean	Range	Mean	Range
<b>Brown</b>				
Ambient	20.90	-0.16 - 29.2	20.97	0.03-29.6
Warmed	23.92	1.91-31.5	23.38	0.68-31.6
<b>Mixed</b>				
Ambient	23.85	0.32-30.7	21.71	0.29-31.0
Warmed	26.47	4.47-33.6	25.20	4.82-33.3
<b>Green</b>				
Ambient	25.15	1.25-35.0	24.51	1.5-35.5
Warmed	27.67	6.62-38.0	27.18	5.44-38.2

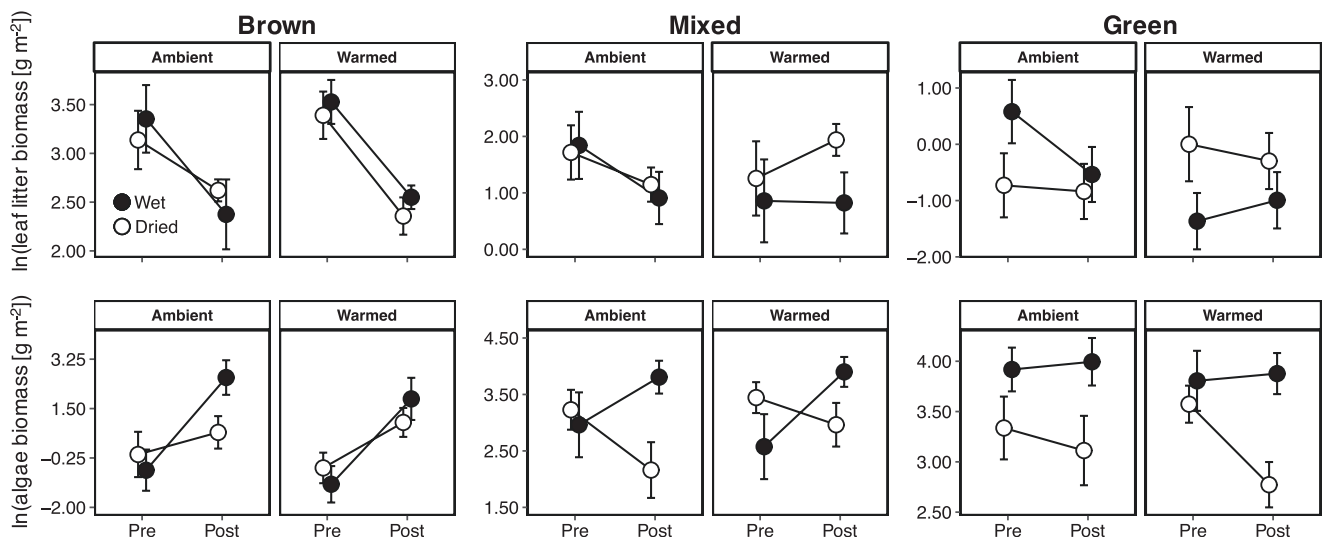


FIGURE 2 Interaction plots of means  $\pm$  standard errors of leaf litter biomass (g AFDM m<sup>-2</sup>) and filamentous algae biomass (g AFDM m<sup>-2</sup>) in wet and dried mesocosms before and after drying. Separate panels depict ambient and warmed mesocosms from different energy channel treatments. Data were natural log transformed and a constant of 0.1 was added to account for zeroes.

higher in the green (mean  $\pm$  SD:  $0.088 \pm 0.074 \text{ g m}^{-2}$ ) mesocosms than in the mixed (mean  $\pm$  SD:  $0.046 \pm 0.051 \text{ g m}^{-2}$ ) and brown (mean  $\pm$  SD:  $0.020 \pm 0.019 \text{ g m}^{-2}$ ) mesocosms. Drying had a negative effect on chlorophyll *a* (Figure 3; Drying  $\times$  Period,  $p < 0.001$ ) but the effect did not differ among energy channels (Table S3). Benthic ash free dry mass (AFDM) ranged from 0.01 to  $19.02 \text{ g m}^{-2}$ , and also differed among energy channels (Table S3). Drying had a marginal negative effect on AFDM (Drying  $\times$  Period,  $p = 0.054$ ) but this effect did not differ among energy channels (Figure 3; Table S3).

### 3.2 | Benthic metabolism

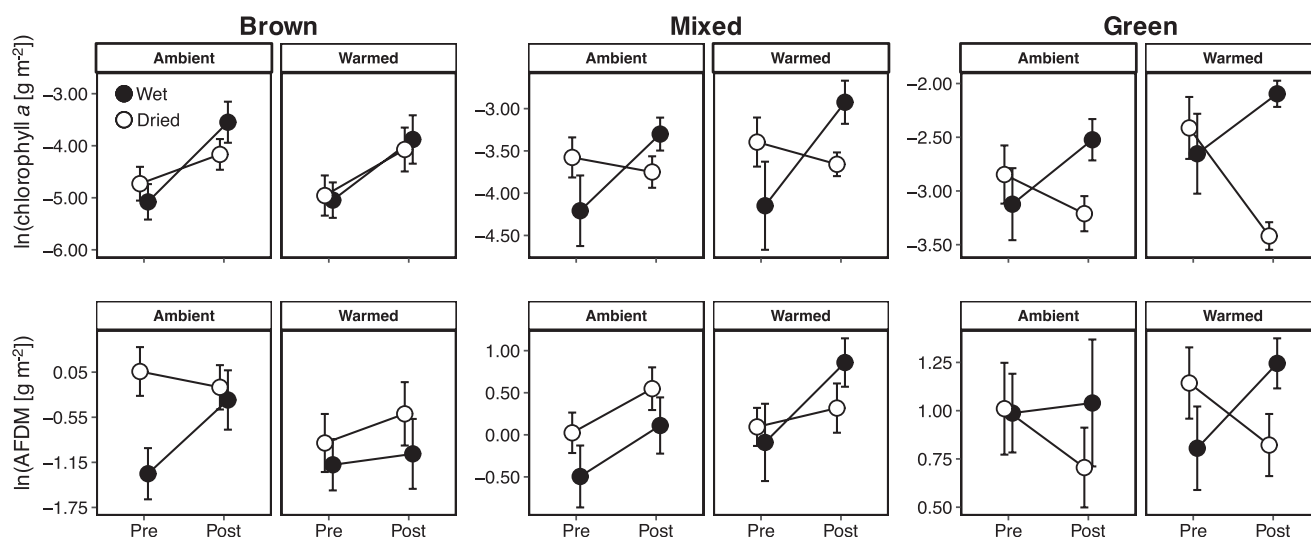
Benthic metabolism was measured on six dates between 21 June and 20 October 2018 (Table S1). We attempted to estimate benthic metabolism on 17 November 2018 but measurements were thwarted by instrument failure. Rates of ER ranged from 0.004 to  $1.170 \text{ mg C m}^{-2} \text{ h}^{-1}$  (mean  $\pm$  SE:  $0.142 \pm 0.012 \text{ mg C m}^{-2} \text{ h}^{-1}$ ) and were not affected by the warming treatment (Table 2). ER did, however, differ among energy channels (Table 2). Mean ER in the green mesocosms (mean  $\pm$  SE:  $0.272 \pm 0.028 \text{ mg C m}^{-2} \text{ h}^{-1}$ ) was approximately 2 times higher than in the mixed mesocosms (mean  $\pm$  SE:  $0.117 \pm 0.015 \text{ mg C m}^{-2} \text{ h}^{-1}$ ), and 4 times higher than in the brown mesocosms (mean  $\pm$  SE:  $0.041 \pm 0.004 \text{ mg C m}^{-2} \text{ h}^{-1}$ ; Figure 4). Drying significantly reduced rates of ER (Figure 4; Drying  $\times$  Period,  $p = 0.002$ ), but this effect did not differ among energy channels (Table 2). GPP ranged from 0.005 to  $1.540 \text{ mg C m}^{-2} \text{ h}^{-1}$  (mean  $\pm$  SE:  $0.305 \pm 0.018 \text{ mg C m}^{-2} \text{ h}^{-1}$ ). Gross primary production was not affected by the warming treatment but differed among energy channels (Table 2). GPP was significantly lower in the brown mesocosms (mean  $\pm$  SE:  $0.131 \pm 0.013 \text{ mg C m}^{-2} \text{ h}^{-1}$ ) than in the mixed (mean  $\pm$  SE:  $0.351 \pm 0.028 \text{ mg C m}^{-2} \text{ h}^{-1}$ ) and green (mean  $\pm$  SE:

$0.431 \pm 0.036 \text{ mg C m}^{-2} \text{ h}^{-1}$ ) mesocosms (Table 2). Overall, drying had a negative effect on GPP (Drying  $\times$  Period,  $p < 0.001$ ), and this effect was consistent across all energy channels (Figure 4; Table 2). During the experiment, NEP ranged from  $-0.177$  to  $0.913 \text{ mg C m}^{-2} \text{ h}^{-1}$  (mean  $\pm$  SE:  $0.162 \pm 0.012 \text{ mg C m}^{-2} \text{ h}^{-1}$ ) and varied among energy channels (Table 2). NEP was unaffected by warming, but drying significantly decreased NEP (Figure 4; Table 2; Drying  $\times$  Period,  $p = 0.032$ ).

## 4 | DISCUSSION

The main objective of this study was to investigate the potential interactive effects of warming and drying on stream metabolism and to examine the importance of energy inputs in modulating those effects. To date, most studies dealing with the effects of climate change on ecosystem metabolism have been less complex, examining responses to only a single stressor (but see Arias Font et al., 2021). Thus, the interactive effects of multiple stressors are largely unknown. We found little evidence of an interactive effect between warming and drying on metabolism, much less a synergistic effect as predicted. However, drying alone led to reductions in benthic organic matter biomass and shifts in the balance of key metabolic processes. Surprisingly, the effects of drying on metabolic processes were similar among energy channel treatments. Thus, energy inputs had little to no influence on the overall response of ecosystem metabolism to stream drying. Overall, the results of this study show that stream drying had a larger effect on metabolic processes than warming and that the effect of drying on stream metabolism may be similar among streams with different energy pathways.

In our study, experimental drying led to diminished standing stocks of filamentous algae, benthic chlorophyll *a*, and benthic



**FIGURE 3** Interaction plots of natural log transformed means  $\pm$  standard errors of benthic chlorophyll *a* ( $\text{g m}^{-2}$ ) and benthic AFDM ( $\text{g AFDM mass m}^{-2}$ ) in wet and dried mesocosms before and after drying. Separate panels depict ambient and warmed mesocosms from different energy channel treatments. Data were natural log transformed and a constant of 0.1 was added to account for zeroes.

**TABLE 2** Summary statistics of averaged linear mixed effects models for individual and interactive effects of warming, energy channel, drying and period on leaf litter biomass, filamentous algae biomass, benthic chlorophyll *a*, benthic Ash free dry mass (AFDM), ecosystem respiration (ER), gross primary production (GPP), net ecosystem production (NEP), and GPP:ER. *p*-values in bold are statistically significant ( $p < 0.05$ ).

Response	Predictor	Estimate	SE	t value	Pr(> t )
Leaf litter biomass	Intercept	2.624	0.231	11.317	<b>&lt;0.001</b>
	Energy channel: Green	-3.375	0.252	13.374	<b>&lt;0.001</b>
	Energy channel: Mixed	-1.521	0.252	6.021	<b>&lt;0.001</b>
	Period: Pre	0.543	0.269	1.665	0.096
	Drying: Not dried	0.040	0.209	0.192	0.847
	Warming: Warmed	0.120	0.263	0.456	0.649
	Not dried × Warming	-0.253	0.412	0.614	0.539
	Pre × Warming	-0.075	0.227	0.330	0.741
	Green × Pre	-0.084	0.270	0.310	0.757
	Mixed × Pre	-0.087	0.276	0.315	0.752
Algae biomass	Intercept	0.928	0.465	1.988	<b>0.047</b>
	Drying: Not dried	1.221	0.222	5.478	<b>&lt;0.001</b>
	Energy channel: Green	1.918	0.273	6.987	<b>&lt;0.001</b>
	Energy channel: Mixed	1.681	0.272	6.150	<b>&lt;0.001</b>
	Period: Pre	-1.447	0.704	1.568	0.117
	Warming: Warmed	-0.024	0.097	0.247	0.805
	Not dried × Pre	-1.454	0.339	4.269	<b>&lt;0.001</b>
	Green × Pre	2.385	0.416	5.706	<b>&lt;0.001</b>
	Mixed × Pre	2.036	0.414	4.894	<b>&lt;0.001</b>
Chlorophyll <i>a</i>	Intercept	-4.233	0.215	19.601	<b>&lt;0.001</b>
	Drying: Not dried	0.597	0.177	3.356	<b>&lt;0.001</b>
	Energy channel: Green	0.990	0.202	4.881	<b>&lt;0.001</b>
	Energy channel: Mixed	0.516	0.176	2.922	<b>0.003</b>
	Period: Pre	-0.546	0.317	1.324	0.185
	Warming: Warmed	0.102	0.092	1.103	0.270
	Not dried × Green	0.407	0.259	1.568	0.117
	Not dried × Mixed	-0.028	0.262	0.107	0.915
	Not dried × Pre	-1.029	0.245	4.192	<b>&lt;0.001</b>
	Green × Pre	1.126	0.259	4.330	<b>&lt;0.001</b>
	Mixed × Pre	0.655	0.259	2.516	<b>0.012</b>
	Green × Not dried × Pre	-0.592	0.461	1.282	0.200
	Mixed × Not dried × Pre	-0.650	0.456	1.420	0.155
AFDM	Intercept	-0.237	0.317	0.745	0.456
	Drying: Not dried	-0.349	0.307	-1.135	0.256
	Energy channel: Green	1.152	0.338	3.402	<b>&lt;0.001</b>
	Energy channel: Mixed	0.506	0.319	1.581	0.114
	Period: Pre	-0.099	0.373	0.205	0.837
	Warming: Warmed	-0.527	0.233	2.256	<b>0.024</b>
	Not dried × Green	0.691	0.302	2.280	<b>0.023</b>
	Not dried × Mixed	0.431	0.302	1.424	0.154
	Not dried × Pre	-0.482	0.249	1.926	0.054
	Not dried × Warmed	0.569	0.302	1.879	0.060
	Green × Pre	0.733	0.302	2.420	<b>0.015</b>
	Mixed × Pre	0.357	0.305	1.169	0.243
	Green × Warmed	-0.250	0.304	0.819	0.413
	Mixed × Warmed	0.293	0.246	1.186	0.236

(Continues)

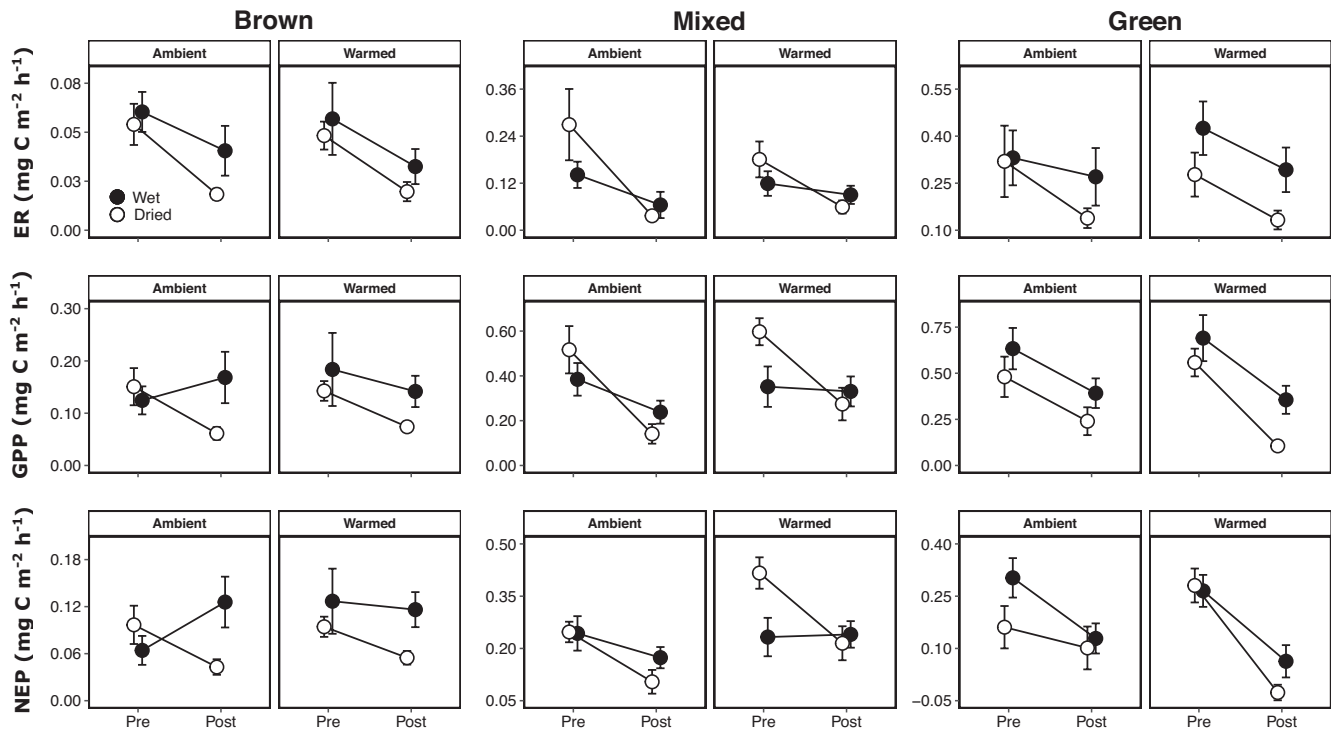
TABLE 2 (Continued)

Response	Predictor	Estimate	SE	t value	Pr(> t )
Ecosystem respiration	Intercept	-4.293	0.250	17.099	<0.001
	Drying: Not dried	0.542	0.176	3.065	0.002
	Energy channel: Mixed	0.868	0.183	4.731	<0.001
	Energy channel: Green	1.847	0.210	8.779	<0.001
	Period: Pre	1.177	0.340	2.446	0.014
	Not dried × Pre	-0.624	0.205	3.032	0.002
	Mixed × Pre	0.110	0.210	0.524	0.600
	Green × Pre	-0.179	0.247	0.723	0.470
	Not dried × Mixed	-0.044	0.170	0.259	0.795
	Not dried × Green	0.149	0.238	0.623	0.533
Gross primary production	Intercept	-2.937	0.254	11.493	<0.001
	Drying: Not dried	0.758	0.176	4.298	<0.001
	Energy channel: Mixed	0.859	0.183	4.680	<0.001
	Energy channel: Green	0.701	0.183	3.813	<0.001
	Period: Pre	0.834	0.346	1.704	0.088
	Warming: Warmed	0.020	0.056	0.348	0.728
	Not dried × Mixed	-0.287	0.211	1.353	0.176
	Not dried × Green	0.318	0.212	1.491	0.136
	Not dried × Pre	-0.945	0.173	5.448	<0.001
	Mixed × Pre	0.020	0.211	2.321	0.020
	Green × Pre	0.589	0.212	2.763	0.006
Net ecosystem production	Intercept	0.045	0.034	1.331	0.183
	Drying: Not dried	0.064	0.029	2.177	0.029
	Energy channel: Mixed	0.077	0.038	1.978	0.048
	Energy channel: Green	-0.022	0.037	0.586	0.558
	Period: Pre	0.035	0.045	0.565	0.572
	Warming: Warmed	0.008	0.028	0.283	0.778
	Not dried × Pre	-0.070	0.032	2.145	0.032
	Mixed × Pre	0.075	0.040	1.836	0.066
	Green × Pre	0.148	0.042	3.520	<0.001
	Not dried × Green	0.010	0.028	0.362	0.718
	Not dried × Mixed	-0.021	0.036	0.591	0.554
	Mixed × Warmed	0.024	0.038	0.631	0.528
	Green × Warmed	-0.020	0.037	0.536	0.592
	Pre × Warmed	0.013	0.029	0.471	0.638
	Not dried × Warmed	-0.007	0.020	0.347	0.729
	Mixed × Pre × Warmed	-0.002	0.018	0.095	0.924
	Green × Pre × Warmed	0.004	0.026	0.167	0.868

AFDM in all energy channel treatments. After rewetting, both filamentous green algal biomass and benthic chlorophyll *a* were ~80% lower in dried mesocosms than in wet mesocosms. Our results corroborate those of other studies. For example, Timoner et al. (2012) demonstrated that drying of a Mediterranean stream initially reduced autotrophic biomass by 80%. In a stream mesocosm experiment, Ledger et al. (2013) found that dewatering reduced algal densities by ~72% and reduced algal crusts by ~80%. Based on our results and those from other studies, it appears that reductions in algal densities and biomass are common in streams and rivers during

and immediately after dry periods. Seeing as the photosynthetic efficiency of stream dwelling autotrophs is positively related to water availability (Timoner et al., 2012), drying should lead to lower rates of GPP and ER. Thus, the loss of water and large reductions in algal biomass during dry periods will have a strong effect on metabolic processes as flow intermittency increases over time.

Reductions in organic matter biomass due to drying led to lower rates of both GPP and ER in dried mesocosms compared to wet mesocosms. Drying also reduced NEP by ~45% in the dried mesocosms relative to the wet mesocosms, supporting our hypothesis



**FIGURE 4** Interaction plots of means  $\pm$  standard errors of benthic chlorophyll *a* ( $\text{g m}^{-2}$ ) and benthic AFDM ( $\text{g AFDM mass m}^{-2}$ ) in wet and dried mesocosms before and after drying. Separate panels depict ambient and warmed mesocosms from different energy channel treatments. Data were natural log transformed and a constant of 1 was added to NEP values account for negative observations.

that stream drying would result in lower rates of NEP. Our results support those from other studies. For example, using a replicated artificial channel experiment, Acuña et al. (2015) demonstrated that non-flow periods differentially affected autotrophic and heterotrophic processes within biofilms, resulting in a shift towards lower P:R ratios (i.e. more heterotrophy). Likewise, community respiration (CR) and NEP decreased significantly during drying in another artificial stream experiment (Zlatanović et al., 2018). Whether reductions in NEP in our experiment were a result of the dissimilarity in responses between autotrophs and heterotrophs is unclear. One important thing to note is that our results partly rely on the assumption that the photosynthetic quotient (PQ) is equal to 1.2. However, PQ has been found to vary spatially, seasonally and even among autotrophic taxa (Soana & Bartoli, 2013). To examine the influence varying PQ has on NEP, we performed a sensitivity analysis using PQ values from 0.4 to 3.0. Overall, NEP decreased with increasing PQ and the percent difference between NEP in wet versus dry mesocosms was also lower at higher PQ values. Nevertheless, dried mesocosms exhibited lower NEPs than wet mesocosms regardless of the PQ used (Figure S2). Therefore, our results and others suggest that increased drying events due to climate change will alter the metabolic balance of streams and rivers, potentially resulting in more heterotrophic ecosystems.

Rates of ER, GPP and NEP differed significantly among energy channel treatments. As expected, the highest rates of GPP occurred in the green energy channel treatment. This was not surprising, given that ecosystem metabolism is largely controlled by light (i.e. PAR)

and resource supply (Bernet et al., 2010; Mulholland et al., 2001). The green mesocosms had high standing stocks of filamentous algae, which likely fueled the high rates of production. Not only did green mesocosms have the highest rates of GPP, they also had the highest rates of ER. In streams with measurable algal production, ER is constrained by GPP over the long term because GPP can supply a significant proportion of the substrate (i.e. autotrophic biomass) for ER (Roman & Sabater, 1999; Yvon-Durocher et al., 2010). In our experiment, both GPP and ER were positively correlated with algal biomass (Figure S3) and GPP and ER were positively correlated with one another (Figure S4). Thus, algal biomass likely acted as a proximal control on stream metabolism in our experiment.

Despite large differences in algal biomass among energy channel treatments, the effects of drying on metabolic processes in those treatments were similar. Therefore, our hypothesis that the green mesocosms would experience greater reductions in NEP was not supported. Why the effects of drying were similar among energy channels remains unclear. One potential mechanism that might explain the similar responses to drying among energy channels is the presence of large, dried algal mats in the green mesocosms. The algal mats, which can be common in small streams of the area, likely acted as shelters that shaded the remaining subsurface water as the mesocosms dried. This could have slowed evaporation and allowed more subsurface water to remain in the green mesocosms than in the brown mesocosms. The additional moisture content could have inhibited or buffered the more severe structural and functional changes that the autotrophic community would have experienced if

the algal mats had not been present (Coulson et al., 2021). Regardless of the mechanism, our results suggest that drying has an overall negative effect on stream metabolism and may result in lower rates of NEP with increased intermittency.

Temperature governs the metabolic processes of respiration and primary production. Therefore, it was somewhat surprising that warming had little effect on metabolic processes (i.e. ER, GPP and NEP) in our experiment. Our hypothesis that experimental warming would increase rates of ER more than GPP and result in lower rates of NEP was not supported. This lack of an effect of warming on metabolic processes contrasts with results from previous studies, including other mesocosm experiments (Hood et al., 2018; Williamson et al., 2016; Yvon-Durocher et al., 2010, 2017). For example, in a mesocosm experiment, Yvon-Durocher et al. (2010) found that warming by ~4°C increased rates of ER faster than rates of GPP, which resulted in a 13% decline in NPP. Why experimental warming had little to no effect on metabolic processes in our experiment is unclear. The temperature response of ER and GPP is determined by complex interactions between temperature and other variables that may covary. For instance, nutrient concentrations, light levels, turbidity, time since last disturbance and even standing stocks of autotrophic biomass can covary with temperature and may inhibit the response of ER and GPP to changes in temperature (Huryn et al., 2014; Michaletz et al., 2014; Padfield et al., 2017). Despite experimental warming having little effect on metabolic processes, both ER and GPP exhibited temperature dependence such that both metabolic processes generally increased with increasing temperature (Figure S5). However, given the variability in metabolic rates across temperatures (Figure S5), it is likely that other variables that may or may not have co-varied with temperature had a greater control over metabolic processes in our experiment.

The lack of a warming effect in our experiment may also be due to the smaller increase in temperature in our experiment (~2.5–3.0°C above ambient temperature) relative to other experiments (increases of 3–5°C; Yvon-Durocher et al., 2010). It is possible that the mesocosms were not warmed enough to produce a large enough response in ER and GPP. Southern Great Plains streams experience a wide range of temperatures, from 0°C in the winter to >40°C in the summer (Erickson & Stefan, 1996). The autotrophic and heterotrophic components of the biofilms and filamentous algae found in our mesocosms are likely adapted to such high temperatures. Furthermore, diminished temperature sensitivity of metabolic processes with increasing temperatures has been observed in a variety of plants and soils (Atkin & Tjoelker, 2003; Fierer et al., 2006). Thus, the relatively small increase in temperature combined with the already warm temperatures experienced by the autotrophic and heterotrophic mesocosm communities during summer may be partially responsible for the lack of warming effect in our experiment. Regardless of the mechanism, other studies have also shown a lack of warming effect on metabolic processes (e.g. Feuchtmayr et al., 2019; Zhou et al., 2018) and the reasons why deserve further investigation.

Given that intermittent streams and rivers may represent more than half of global stream network length (Acuña et al., 2014; Messenger

et al., 2021) and that more rivers worldwide are drying due the effects of climate change and water abstraction (Zipper et al., 2021), understanding how such drying events affect the sequestration and remineralization of carbon is of utmost importance. Our results highlight the importance of recognizing that stream drying-rewetting cycles can have a strong influence on the sequestration and respiration of carbon in river networks. Yet, our experiment consisted of one single drying event that lasted for 24 days. The duration, frequency and magnitude of drying events is predicted to increase as the climate continues to change (Moidu et al., 2021). Conversely, streamflows in some parts of the world are predicted to become more stable or even increase (Ahiablame et al., 2017). The changes in flow regimes in the face of climate change are complex, and the changes to the metabolic balance of rivers and streams are likely to vary worldwide. Nevertheless, our experiment suggests that stream drying may significantly alter the metabolic balance of many aquatic ecosystems and could result in an overall decline in NEP.

#### AUTHOR CONTRIBUTIONS

Daniel Nelson and Daniel C. Allen conceived and designed the study. All authors conducted fieldwork; Daniel Nelson analyzed the data and led the writing with contributions from Michelle H. Busch, Darin A. Kopp and Daniel C. Allen.

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#### CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. Daniel Allen is an Associate Editor of Functional Ecology, but took no part in the peer review and decision-making process for this paper.

#### DATA AVAILABILITY STATEMENT

Data are available from the primary author upon reasonable request and from the Knowledge Network for Biocomplexity (KBN) repository <https://doi.org/10.5063/F1707ZWH> (Nelson et al., 2023).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Appendix S1.** Mesocosm configuration and set-up.

**Table S1.** Timeline of significant events that occurred during the experiment.

**Table S2.** List of the fixed effects structure of linear mixed effects sub-models with  $\Delta AIC < 2$  for each dependent variable.

**Figure S1.** Mean biomass of filamentous algae in each energy channel treatment over the course of the experiment.

**Figure S2.** Boxplots showing the effect that varying photosynthetic quotients (PQs) have on net ecosystem production (NEP).

**Figure S3.** Relationship between ecosystem respiration (ER) and algal biomass and gross primary production (GPP) and algal biomass.

**Figure S4.** Relationship between ecosystem respiration (ER) and gross primary production (GPP).

**Figure S5.** Relationship between ecosystem respiration (ER), gross primary production (GPP), net ecosystem production (NEP) and temperature.

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