

## LETTER

**Nutrient enrichment intensifies the effects of warming on metabolic balance of stream ecosystems**Wyatt F. Cross <sup>1†\*</sup>, James M. Hood <sup>2†\*</sup>, Jonathan P. Benstead <sup>3</sup>, Alexander D. Hury, <sup>3</sup> Jill R. Welter, <sup>4</sup> Gísli M. Gíslason <sup>5</sup>,  
Jón S. Ólafsson <sup>6</sup><sup>1</sup>Montana State University, Department of Ecology, Bozeman, Montana; <sup>2</sup>The Ohio State University, Department of Evolution, Ecology, and Organismal Biology, Translational Data Analytics Institute, and Aquatic Ecology Laboratory, Columbus, Ohio; <sup>3</sup>University of Alabama, Department of Biological Sciences, Tuscaloosa, Alabama; <sup>4</sup>Department of Biology, Saint Catherine University, St. Paul, Minnesota; <sup>5</sup>Institute of Life and Environmental Sciences, University of Iceland, Reykjavík, Iceland; <sup>6</sup>Institute of Marine and Freshwater Fisheries, Reykjavík, Iceland**Scientific Significance Statement**

Streams and rivers contribute substantially to the global carbon cycle by producing and transforming organic matter and releasing large amounts of carbon dioxide to the atmosphere. Although recent studies show that warming is likely to favor the mineralization of organic matter over its production, and thus increase the contribution of streams to atmospheric carbon dioxide, it is still unknown whether anthropogenic nitrogen and phosphorus enrichment is buffering or intensifying this effect. We provide whole-stream experimental evidence that nutrient enrichment interacts with warming to amplify carbon mineralization and thus increase the contribution of streams to carbon dioxide in the atmosphere.

**Abstract**

Climate warming and eutrophication are leading drivers of environmental change, and both are likely to alter the role of freshwaters in the global carbon cycle. Recent studies demonstrate that warming of streams can increase freshwater contributions of CO<sub>2</sub> to the atmosphere, yet little is known about how such contributions are modulated by the identity or supply of limiting nutrients. We quantified responses of ecosystem metabolism and metabolic balance to whole-stream enrichments of either nitrogen or phosphorus across a 5°C range of ambient temperature. We show that nutrient enrichment drove large reductions in net ecosystem production, and interactions with temperature amplified these effects in warm N-enriched streams. Partitioning of autotrophic and heterotrophic respiration revealed that these responses were driven by increased respiration of microbial heterotrophs. Our study provides direct experimental evidence that warming-induced increases in CO<sub>2</sub> emissions to the atmosphere are likely intensified in stream ecosystems subject to eutrophication.

\*Correspondence: [wyatt.cross@montana.edu](mailto:wyatt.cross@montana.edu); [hood.211@osu.edu](mailto:hood.211@osu.edu)**Associate editor:** James B. Heffernan**Author Contribution Statement:** WFC and JMH co-led the manuscript and contributed equally. WFC, JMH, JPB, ADH, and JRW conceived the study and approach. WFC, JMH, JPB, and ADH, and JRW conducted experiments and collected the data. WFC and JMH analyzed the data. WFC and JMH wrote the manuscript and all authors contributed to additional drafts and revision.

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**Data Availability:** All data and code used in this study are available at <https://github.com/hood211/IceMetab2.git>.

Additional Supporting Information may be found in the online version of this article.

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## Introduction

Inland waters produce, transport, store, and mineralize large amounts of organic matter and collectively release over two petagrams (Pg;  $10^{15}$  g) of carbon as  $\text{CO}_2$  to the atmosphere annually (Cole et al., 2007; Battin et al., 2009; Raymond et al., 2013). Although a large fraction of this carbon originates in terrestrial ecosystems (Battin et al. 2008; Drake et al., 2018), the combined metabolism of autotrophic and heterotrophic aquatic organisms nonetheless plays a significant role in carbon processing and flux to the atmosphere (Hotchkiss et al., 2015; Demars et al., 2020; Rocher-Ros et al., 2020). Understanding how the net activity of gross primary production (GPP) and ecosystem respiration (ER; i.e., the ‘metabolic balance’) responds to environmental change thus has significant implications for food-web dynamics (i.e., autotrophic vs. heterotrophic state; Dodds & Cole, 2007) and carbon-cycling at regional and global scales.

Climate warming and nutrient enrichment are leading drivers of environmental change in freshwater ecosystems (Jeppesen et al., 2010; Moss et al., 2011), and both may influence the role of freshwaters in the carbon cycle (Battin et al., 2009; Li et al., 2021). Warming alters biological  $\text{CO}_2$  production and sequestration through its direct effects on physiological rates and through its influence on community composition and biomass (Yvon-Durocher et al., 2010a,b). In rivers, recent work has demonstrated that  $1^\circ\text{C}$  of warming could lead to a 24% reduction in net ecosystem production (NEP) at a global scale (Song et al., 2018), and hence greater contributions of  $\text{CO}_2$  to the atmosphere. This result is generally supported by other studies in streams (Demars et al., 2011), ponds (Yvon-Durocher et al., 2017), and lakes (Kraemer et al., 2017), especially in ecosystems where a substantial fraction of ER is driven by heterotrophs (Demars et al., 2016). However, uncertainty remains about the relative importance of nutrient supply (Bernhardt et al., 2018, 2022), and particularly how nutrients interact with warming to alter metabolic balance (Heffernan, 2018).

Changes in nitrogen (N) and phosphorus (P) availability influence photosynthetic and respiratory processes by alleviating or exacerbating elemental limitation and may thus modify ecosystem responses to temperature (Cross et al. 2015). Pervasive eutrophication of lakes and wetlands (Carpenter et al., 1998) enhances ecosystem metabolism via positive effects on algal and bacterial growth (Hanson et al., 2003; Hoellein et al., 2013; Solomon et al., 2013), with varying effects on metabolic balance (del Giorgio & Peters, 1994; Cole et al., 2000; Scharfenberger et al., 2019). By contrast, less is known about how nutrients modify ecosystem metabolism of rivers (Arroita et al., 2019; Myrstener et al., 2021), and particularly how such responses may interact with warming (Guðmundsdóttir et al., 2011). Given the important role of streams and rivers in the global carbon cycle (Cole et al., 2007; Butman & Raymond, 2011), and the ubiquity of

nutrient-enriched rivers (McDowell et al., 2020), studies that examine interactions between warming and nutrient supply are required to understand combined responses to these dominant global-change drivers.

We quantified ecosystem metabolism and metabolic balance in response to whole-system nutrient amendments in four sub-arctic streams distributed along a natural thermal gradient. We manipulated N and P in successive years to assess how each nutrient separately interacts with temperature to influence ecosystem metabolism. We predicted that additions of both N and P would lead to increases in metabolism of autotrophs and heterotrophs, but that greater heterotrophic responses would reduce metabolic balance and amplify  $\text{CO}_2$  production. In addition, we expected these responses would be most pronounced at higher temperatures, given the generally steeper temperature dependence of respiratory vs. photosynthetic reactions (Allen et al., 2005). Finally, based on low dissolved N:P ratios, previous experimental results (Friberg et al., 2009), and the presence of  $\text{N}_2$ -fixers in our study streams (Williamson et al., 2016), we predicted a stronger response to N than to P addition.

## Methods

We conducted experimental N and P additions to four Icelandic headwater streams ( $64^\circ 03' \text{N}$ ;  $21^\circ 18' \text{W}$ ) distributed along a thermal gradient ( $9.7^\circ\text{C}$  –  $15.1^\circ\text{C}$ ). Differences in ambient temperature result from indirect geothermal heating of soil and bedrock (Árnason et al., 1969); however, streams were similar in other physiochemical characteristics (O’Gorman et al., 2012), including low dissolved N and P concentrations (Supplementary Fig. 1 and Supplementary Table 1). Experimental N and P treatments were employed over three successive years between May and August, with ambient nutrient conditions in 2015, P addition in 2016 ( $\text{H}_3\text{PO}_4$ ; target concentration:  $200 \mu\text{g P L}^{-1}$ ,  $\sim 20$ -times ambient), and N addition in 2017 ( $\text{NH}_4\text{NO}_3$ ; target concentration:  $200 \mu\text{g N L}^{-1}$ ,  $\sim 20$ -times ambient; Supplementary Fig. 1; Supplementary Methods). In each year, we quantified metabolic responses during July–August, a period of peak biomass and productivity when high flows were less frequent.

To estimate daily ecosystem metabolism, including GPP, ER, and NEP ( $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ), we used the two-station open-channel method (Hall & Hotchkiss, 2017). This method required detailed measurement of stream and groundwater dissolved oxygen concentrations, air-water gas exchange, temperature, discharge, light, depth, and streambed area (Supplementary Methods). We employed Bayesian inverse modeling to fit a model of dissolved oxygen to data and estimate posterior distributions of ER, groundwater dissolved oxygen exchange, air-water gas exchange, and photosynthesis-irradiance model parameters (Holtgrieve et al., 2010; Hall et al., 2016; Supplementary Methods). Daily estimates of GPP,

ER, and NEP were converted from units of oxygen to C using a photosynthetic quotient of 1.2 and a respiratory quotient of 0.8 (Hall and Hotchkiss, 2017). Metabolism modeling was conducted using R (R Core Team, 2020).

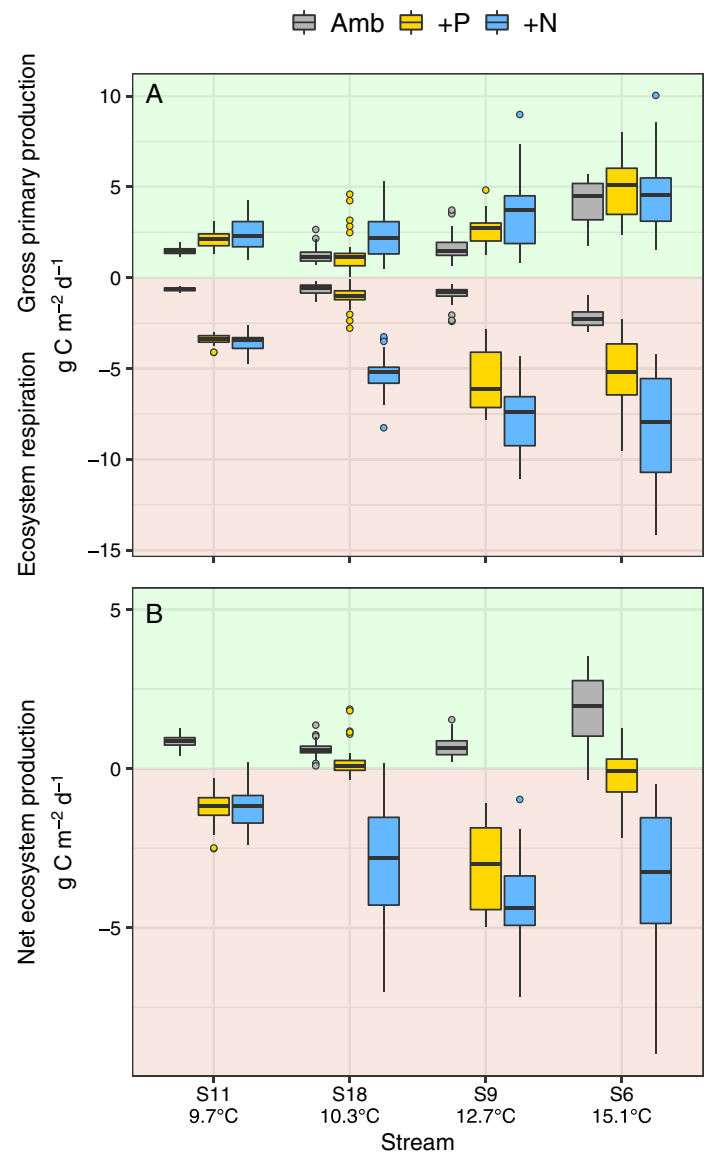
**Statistical approach.** We used model-selection (Burnham and Anderson, 2002) to examine metabolic responses to temperature and nutrient enrichment. We built generalized additive models (GAMs; Wood, 2017; Zuur and Ieno, 2018) for GPP, ER, and NEP in three data subsets: the ambient year, ambient and P-enrichment years, and ambient and N-enrichment years (Supplementary Methods). The most likely models were identified based on  $AIC_C$ ; we considered models with a  $\Delta AIC_C < 2$  as having substantial empirical support and models with a  $\Delta AIC_C$  between 4 and 7 as having less support. GAM models were constructed with the *gamm* function using *mgcv* in R (version 4.1.0).

To quantify the contributions of autotrophic vs. heterotrophic metabolism to ecosystem respiration, we used the framework of Hall and Beaulieu (2013). Their model assumes that autotrophic respiration (and that of closely-associated heterotrophs) represents the lower limit of daily ER and can be approximated by the 90% quantile of the relationship between daily GPP and ER. The remainder of ecosystem respiration is assumed to derive from heterotrophic organisms (Supplementary Methods). To assess differences in the heterotrophic contribution to ecosystem respiration among years, we compared overlap in 95% confidence intervals.

## Results

Under ambient nutrient conditions (average soluble reactive phosphorus [SRP]:  $11 \mu\text{g L}^{-1}$ ; average  $\text{NO}_3 + \text{NH}_4\text{-N}$ :  $10 \mu\text{g L}^{-1}$ ) and low dissolved N:P ratios (average molar N:P ratio: 1.9; Supplementary Fig. 1), mean stream temperature had a positive effect on GPP and ER, and both fluxes had similar temperature dependences (Fig. 1(A) and Table 1). A generalized additive model (GAM) that contained temperature provided the most likely model, but a model with stream identity had substantial support (Table 1 and Supplementary Fig. 2). Net ecosystem production (NEP) was uniformly positive at ambient nutrient concentrations (Fig. 1(B)), suggesting net autotrophy in all streams at low nutrient supply and during peak growing season. NEP was also highest in the warmest stream, and had a temperature dependence that was similar to GPP and ER (Fig. 1(B) and Table 1). A GAM for NEP that included temperature had more support than a model that included stream identity (Table 1).

Phosphorus enrichment stimulated both GPP and ER (Fig. 1(A)), but this effect was not influenced by stream temperature. The positive response to P was 10 times greater for ER (average 298% increase) than for GPP (32% increase), and the magnitude of these differences was not related to mean stream temperature (Fig. 2 and Supplementary Fig. 2). The most likely GAM for GPP contained additive effects of stream



**Fig. 1.** (A) Gross primary production ( $\text{g C m}^{-2} \text{d}^{-1}$ ) and ecosystem respiration ( $\text{g C m}^{-2} \text{d}^{-1}$ ) and (B) net ecosystem production ( $\text{g C m}^{-2} \text{d}^{-1}$ ) in the study streams under ambient nutrient concentrations (gray) and during phosphorus (yellow) or nitrogen (blue) enrichment. Boxes show the median and 25<sup>th</sup> or 75<sup>th</sup> quantiles, whiskers extend to 1.5 times the interquartile range, and points represent data outside of this range.

identity and P-enrichment, demonstrating that the stimulatory effect of P did not differ among streams (Table 1). In contrast, the most likely GAM for ER contained an interaction between stream identity and P-enrichment, reflecting the much smaller effect of P in the 10.3°C stream (60% increase) relative to the other streams (up to a 420% increase). A GAM that included mean stream temperature was supported for GPP, but the inclusion of temperature was not supported in models of ER (Table 1). The asymmetrical responses of GPP and ER to P enrichment led to reductions in NEP at all

**Table 1.** Generalized additive models (GAM) fit to the metabolism time series for ambient, ambient and phosphorus-enriched, and ambient and nitrogen-enriched datasets.

Treatment	Metabolic component	Model	Temperature dependence (eV)	Df	AICc	$\Delta$ AICc	$R^2_{adj}$
Ambient	GPP	Temp	1.37 (0.38)	7	-38.1	0.00	0.55
		Stream	--	9	-37.6	0.52	0.70
	ER	Temp	1.63 (0.36)	7	-0.7	0.00	0.61
		Stream	--	9	1.5	2.21	0.67
	NEP	Temp	1.34 (0.84)	6	80.6	0.00	0.37
		Stream	--	9	84.5	3.86	0.51
Phosphorus	GPP	Stream + P	--	10	-25.8	0.00	0.58
		Temp + P	1.52 (0.37)	8	-25.1	0.70	0.45
	ER	Stream $\times$ P	--	13	-24.1	0.00	0.82
		Temp + P	1.62 (0.49)	8	-18.9	5.20	0.61
	NEP	Stream $\times$ P	--	13	291.6	0.00	0.79
		Temp $\times$ P	Ambient: 1.32 +P: -0.38 <sup>†</sup>	9	298.7	7.16	0.45
Nitrogen	GPP	Temp + N	1.28 (0.28)	8	-35.3	0.00	0.54
		Stream + N	--	10	-33.8	1.50	0.59
	ER	Temp + N	1.36 (0.26)	8	-123.1	0.00	0.87
		Stream + N	--	10	-118.9	4.15	0.87
	NEP	Temp $\times$ N	Ambient: 1.39 +N: -2.94 <sup>‡</sup>	9	618.0	0.00	0.75
		Stream $\times$ N	--	13	623.0	5.07	0.79

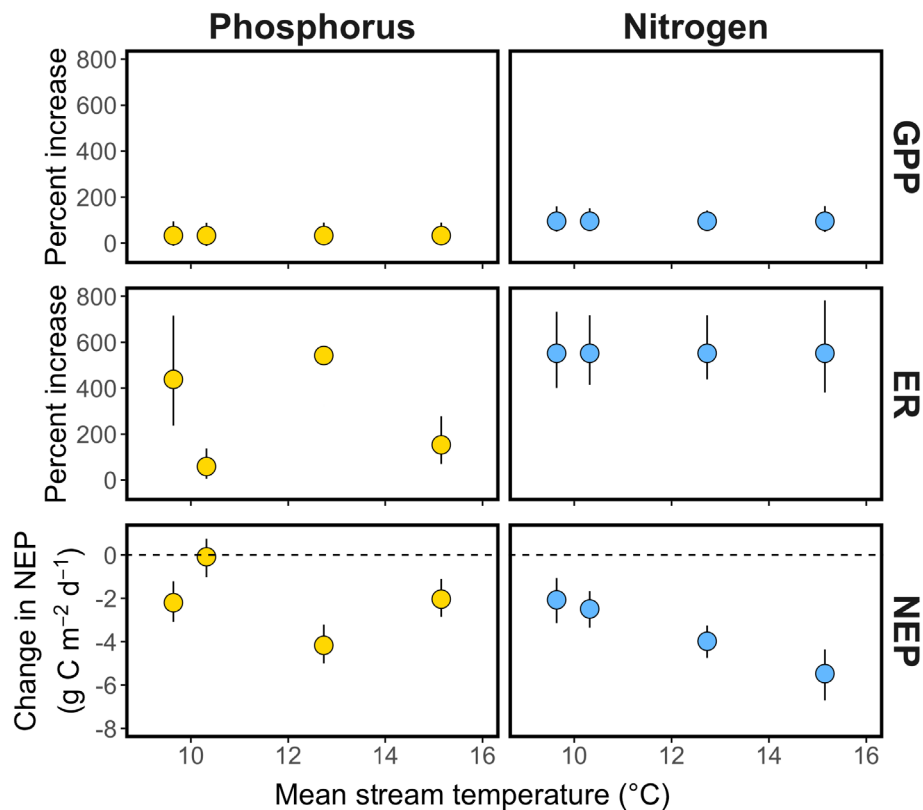
Models were fit to GPP, ER, and NEP time series and compared using the Akaike Information Criterion corrected for small sample sizes (AICc). Delta AICc is the difference between each model and the model with the lowest AIC. Here we present models with the most support, as well as models based on a priori hypotheses to assess interactions between temperature and nutrient enrichment. Stream is a categorical variable; 'Temp' is mean inverse stream temperature; 'P' and 'N' signify the phosphorus and nitrogen treatments, respectively. Light was retained as a covariate in all models. The  $R^2_{adj}$  value represents the proportion of total variation explained by the model. Temperature dependences are  $-1$  times the temperature coefficient ( $\pm 1$  standard error) and reported as electron volts (eV). Models with temperature  $\times$  nutrient interactions show the temperature coefficients for both ambient and nutrient-enriched conditions. To account for temporal autocorrelation, all models contained an autoregressive integrated moving average model of order 1 with a moving average over 1. <sup>†</sup>The standard error for the temperature effect was 1.93 and for the temperature  $\times$  P interaction was 1.74. <sup>‡</sup>The standard error for temperature effect was 1.23 and for the temperature  $\times$  N interaction was 1.

temperatures (Fig. 1(B), Fig. 2, and Supplementary Fig. 2). In three of the four study streams, the metabolic balance during July–August shifted from net autotrophy to net heterotrophy in response to elevated P (i.e., positive to negative NEP; Fig. 1(B)). The most likely GAM for NEP included an interaction between stream identity and P-enrichment, while a model that included mean stream temperature had limited support (Table 1).

Nitrogen enrichment strongly affected ecosystem metabolism, with large positive effects on both GPP (average 95% increase) and ER (average 552% increase) relative to ambient conditions (Fig. 1(A) and Fig. 2). These fluxes were also positively influenced by mean stream temperature, but the proportional response to N-enrichment was relatively consistent among streams of varying temperature (Fig. 2). GAMs that included both N-enrichment and mean stream temperature provided the best fit to the GPP and ER data (Table 1), and the temperature dependences of these fluxes were relatively

similar to those found under ambient nutrient conditions (Table 1 and Supplementary Fig. 2). In contrast, N-enrichment and temperature interacted to influence the balance of GPP and ER, leading to large reductions in NEP that shifted streams from net autotrophy to net heterotrophy (Fig. 1(B)). This shift to heterotrophy was amplified with warming, demonstrated by a more than doubling of the N-enrichment effect over the  $\sim 5^\circ\text{C}$  gradient (i.e., from a difference of  $2.1 \text{ g C m}^2 \text{ d}^{-1}$  at  $9.7^\circ\text{C}$  to  $5.5 \text{ g C m}^2 \text{ d}^{-1}$  at  $15.1^\circ\text{C}$ ; Fig. 1(B), Fig. 2, Supplementary Fig. 2). The mostly likely GAM for NEP included an interaction between N-enrichment and mean stream temperature (Table 1). Model parameters demonstrated that NEP increased with stream temperature under ambient conditions (31% increase per  $^\circ\text{C}$ ), but this pattern was reversed during N-enrichment (29% decrease per  $^\circ\text{C}$ ; Table 1 and Supplementary Fig. 2).

The large effects of both N and P enrichment on carbon mineralization motivated further analysis to quantify the



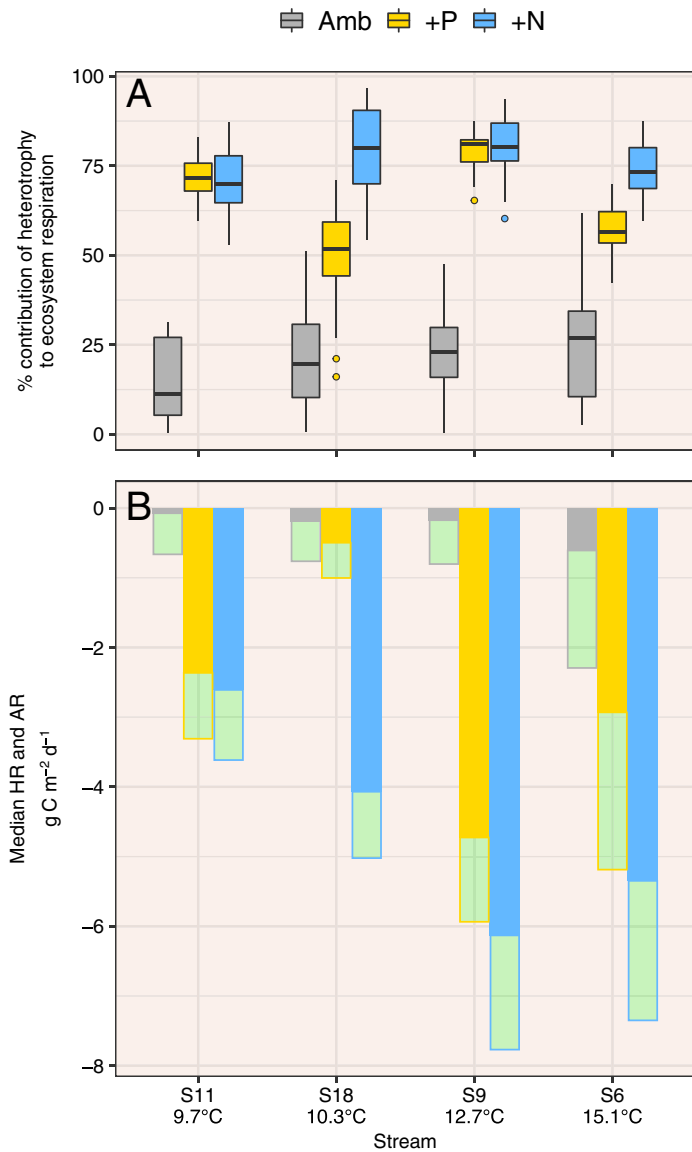
**Fig. 2.** Effect sizes representing GPP, ER, and NEP responses to phosphorus and nitrogen enrichment across a 5°C temperature gradient. Effect sizes for GPP and ER are the GAM-predicted mean percent increase during the treatment relative to ambient conditions. Effect sizes for NEP were calculated as the absolute difference between the treatment and ambient years. We fixed light at median values to aid in visualization of temperature effects in the absence of other covariates. Error bars (sometimes obscured by the point) represent 90% credible intervals generated by randomly sampling from a normal distribution characterized by the ambient and treatment GAM-predicted fit and standard error.

relative contributions of autotrophic vs. heterotrophic organisms in driving these responses. Following Hall and Beaulieu (2013), we conservatively assumed that 44% of daily GPP is respired immediately by autotrophs (see Supplementary Methods for sensitivity analysis). We found that increased carbon release in response to both N and P enrichment was overwhelmingly driven by heterotrophic metabolism. At ambient nutrient concentrations, only a small proportion (range of median values: 11–27%) of ER could be attributed to heterotrophs, while the majority (73–89%) of ER was associated with primary producers (i.e., algae, aquatic plants, and cyanobacteria) that maintained net autotrophy (Fig. 3). In contrast, under N-enriched conditions, most of the metabolic activity (range of median values: 70–80%) was attributed to heterotrophs (i.e., archaea, bacteria, fungi) that were responsible for the switch to net heterotrophy and elevated CO<sub>2</sub> production, particularly in warm streams (Fig. 3; non-overlapping 95% CIs between ambient and N treatments). These responses were robust to the assumed fraction of GPP respired by autotrophs (23–63%; Supplementary Fig. 3). Under P-enriched conditions, the results were qualitatively similar: most of the

increased ER was driven by heterotrophs (Fig. 3). This response during P-enrichment was slightly less robust, as the 95% confidence intervals of ambient and P treatments overlapped in two of the four streams.

### Discussion

We found that N and P enrichment had a large effect on metabolic balance of the study streams, with increases in ER that were of much greater magnitude than those driven by warming alone. Across all temperatures, NEP was reduced by an average of 2.1 and 3.5 g C m<sup>-2</sup> d<sup>-1</sup> in response to N and P enrichment, respectively, equating to an average decline in NEP of roughly 300%. To put this in context, Song et al. (2018), leveraging a large compilation of stream metabolism data, estimated that 1°C of warming is likely to reduce average NEP by up to 24%, leading to an additional 0.02 Pg y<sup>-1</sup> of carbon emitted by running waters globally. Although Song et al.'s analysis encompassed a relatively broad range of trophic status, it did not explicitly address the role of nutrient supply on metabolism. In addition, we may have



**Fig. 3.** (A) Percent (%) contribution of heterotrophic organisms to total ecosystem respiration in the study streams under ambient nutrient concentrations (gray) and during phosphorus (yellow) or nitrogen (blue) enrichment. Boxes show the median and 25<sup>th</sup> or 75<sup>th</sup> quantiles, whiskers extend to 1.5 times the inter-quantile range, and points represent data outside of this range. (B) Total ecosystem respiration ( $\text{g C m}^{-2} \text{d}^{-1}$ ) partitioned by heterotrophic (HR; gray, yellow, or blue) and autotrophic (AR; light green) contributions.

underestimated ecosystem respiration because autotrophs may have higher respiration during the day than at night (Hotchkiss & Hall, 2014). In light of our results, it is thus likely that projections of global carbon cycling by streams in response to warming will significantly underestimate  $\text{CO}_2$  production and emissions if effects of nutrient enrichment are not considered.

The influence of nutrient enrichment on stream metabolic balance depended on temperature, i.e., we detected the largest

reduction in NEP under warm N-rich conditions. Our study thus provides evidence that nutrient enrichment may exacerbate warming-induced release of  $\text{CO}_2$  from streams to the atmosphere. Running water ecosystems may be unusual in this regard. For instance, previous research in lakes and ponds has shown that enrichment may increase NEP (del Giorgio & Peters, 1994; Cole et al., 2000), which is counter to – and may partially balance – reductions in NEP driven by warming. Similarly, carbon sequestration in forest ecosystems is positively influenced by nutrient availability (Fernández et al., 2014), and elevated nutrients on land may lessen any short or long-term increase in carbon release driven by climate warming (Allison et al., 2010). Therefore, unlike in lakes and forests, increased nutrient supply to streams will likely amplify carbon loss (Rosemond et al., 2015) and  $\text{CO}_2$  emissions, instead of acting as a buffer to the carbon release driven by environmental warming. Recent studies focused on methane in lakes, a more potent greenhouse gas, also show that both warming and eutrophication can enhance methane release, suggesting that additional work is needed to characterize how these factors influence metabolic balance under different redox conditions (Davidson et al., 2018; Sepulveda-Jauregui et al., 2018; Li et al., 2021).

Our study also suggests that predicting the combined effects of warming and nutrient supply on the metabolic balance of ecosystems will benefit from a deeper understanding of nutrient status and limitation of major trophic categories (i.e., autotrophs vs. heterotrophs; Dodds & Cole, 2007; López-Urrutia & Moran, 2007), as well as the net effect of their potentially divergent responses to nutrients on carbon cycling (Yvon-Durocher et al., 2010a). Although eutrophication may manifest as a consequence of runaway primary producer growth and nighttime oxygen depletion driven by autotrophic respiration, it is possible that oxygen depletion is driven by the release of heterotrophic microbes from nutrient limitation (Elwood et al., 1981; Suberkropp et al., 2010) and elevated heterotrophic respiration in the presence of decaying primary producers and abundant particulate or dissolved detritus (Mallin & Cahoon, 2020). Further, because cellular respiration responds more strongly to temperature than does photosynthesis (Allen et al., 2005), heterotrophic contributions to metabolic balance are likely to be amplified by warming. Our analysis demonstrated that large increases in ER in response to both N and P enrichment were not due to increased autotrophic respiration, and that mineralization of stored or dissolved organic carbon by heterotrophic bacteria was principally responsible for reduced NEP and accelerated  $\text{CO}_2$  production. This finding is noteworthy because assessment of eutrophication and general understanding of trophic status in freshwaters has been built largely upon assays that implicitly or explicitly focus on responses of primary producers and may not therefore provide a reliable basis for predicting changes in whole-ecosystem metabolic balance (Dodds, 2007; Elser et al., 2007; Ardón et al., 2020).

One question raised by our results is whether the nutrient-induced increases in heterotrophic respiration could be sustained in the long term by carbon subsidies or would instead decline when the primary sources of carbon – i.e., dissolved organic carbon (DOC) and particulate carbon stored within sediments – became limiting. To evaluate this question, we compared the maximum flux of heterotrophic respiration in our study ( $6.1 \text{ g C m}^{-2} \text{ d}^{-1}$  in S9 during N enrichment) to measurements of DOC flux and carbon storage in three of the study streams for which data are available. Given their average DOC concentration, discharge, and stream area, we calculated a mean flux of  $87 \text{ g DOC m}^{-2} \text{ d}^{-1}$  (Supplementary Table 2), a quantity far greater than the maximum heterotrophic respiration we estimated. In addition, the streams likely store a substantial amount of particulate detrital carbon based on previous surficial benthic samples (Supplementary Table 2; Junker et al., 2020). Given these comparisons, there appears to be sufficient carbon to sustain elevated heterotrophic respiration in the long term, even if only a fraction of this subsidized material is bioavailable. However, it is important to note that the amount, quality, and timing of carbon subsidies could also shift in response to changing climate and the resulting mobilization of carbon and nutrients at large scales (e.g., de Wit et al., 2016). It is therefore possible that the magnitude of responses measured in our study could increase or decrease over time. Moreover, if respiration of particulate carbon was favored over DOC, the storage of streambed carbon could be depleted over long time scales.

Our study system provided the benefit of a ‘natural warming experiment’, affording a realistic view into how nutrient enrichment can interact with temperature as streams warm. Yet, the extent to which our results can be generalized depends on whether our sites represent streams and rivers more broadly. The study streams are perhaps most analogous to those that drain open-canopied grasslands and shrublands, alpine and tundra environments, and non-forested landscapes with minimal riparian vegetation and low inputs of allochthonous particulate organic matter (Petersen et al., 1995). Together, these vegetative classes cover more than half of the terrestrial surface (Dodds, 1997), suggesting that the direction and magnitude of our results may be representative of a large fraction of streams globally. In forested streams we might expect an even stronger reduction in NEP under warm, nutrient-rich conditions, given that heterotrophic processes dominate and GPP tends to be low in such ecosystems (Mulholland et al., 2001; Manning et al., 2018). Nonetheless, there is still much to be learned about how warming and nutrient enrichment influence metabolic balance among streams of different size, background nutrient concentrations, food-web structure, and flow regime.

We found strong effects of both N and P enrichment on ecosystem metabolism, but the magnitude of heterotrophic vs. autotrophic responses differed between these elements. Most notably, P-enrichment had only a weak stimulatory effect on GPP relative to N, but a comparably large effect on

ER in three of the four study streams. These differences suggest that release from P limitation within some component of the heterotrophic microbial community was responsible for the large increase in ER. Increased heterotrophic respiration in response to N-enrichment also suggests the potential for dual-nutrient limitation of these heterotrophic communities, perhaps through differences in nutrient limitation among microbial taxa. Interestingly, Peterson et al. (1985) showed that P-enrichment of an arctic river had a larger effect on autotrophic vs. heterotrophic metabolism, but this study was predominantly focused on the activity on biofilm communities rather than the collective metabolism of the whole river. Although there is a growing recognition that different taxa, trophic groups, or ecosystem components may be limited by multiple elements, there are few demonstrations of this at the whole-ecosystem level (Sundareshwar et al., 2003). Moving forward, such results must be considered broadly in stream management or restoration efforts (e.g., water treatment, agricultural practices, riparian restoration) that have historically focused on single elements, as in wastewater treatment which has favored removal of P over N (Lewis et al., 2011; Tong et al., 2020), and which may unintentionally enhance CO<sub>2</sub> emission from streams and its sensitivity to warming.

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