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Multiple stressors alter greenhouse gas concentrations in streams through local and distal processes

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Abstract

Streams are significant contributors of greenhouse gases (GHG) to the atmosphere, and the increasing number of stressors degrading freshwaters may exacerbate this process, posing a threat to climatic stability. However, it is unclear whether the influence of multiple stressors on GHG concentrations in streams results from increases of in-situ metabolism (i.e., local processes) or from changes in upstream and terrestrial GHG production (i.e., distal processes). Here, we hypothesize that the mechanisms controlling multiple stressor effects vary between carbon dioxide (CO_2) and methane (CH_4), with the latter being more influenced by changes in local stream metabolism, and the former mainly responding to distal processes. To test this hypothesis, we measured stream metabolism and the concentrations of CO_2 (pCO_2) and CH_4 (pCH_4) in 50 stream sites that encompass gradients of nutrient enrichment, oxygen depletion, thermal stress, riparian degradation and discharge. Our results indicate that these stressors had additive effects on stream metabolism and GHG concentrations, with stressor interactions explaining limited variance. Nutrient enrichment was associated with higher stream heterotrophy and pCO_2, whereas pCH_4 increased with oxygen depletion and water temperature. Discharge was positively linked to primary production, respiration and heterotrophy but correlated negatively with pCO_2. Our models indicate that CO_2-equivalent concentrations can more than double in streams that experience high nutrient enrichment and oxygen depletion, compared to those with oligotrophic and oxic conditions. Structural equation models revealed that the effects of nutrient enrichment and discharge on pCO_2 were related to distal processes rather than local metabolism. In contrast, pCH_4 responses to nutrient enrichment, discharge and temperature were related to both local metabolism and distal processes. Collectively, our study illustrates potential climatic feedbacks resulting from freshwater degradation and provides insight into the processes mediating stressor impacts on the production of GHG in streams.

KEYWORDS
carbon dioxide, cumulative stressor effects, ecosystem functioning, eutrophication, global change, greenhouse gas emissions, hypoxia, land-use intensification, methane, stream metabolism

1 | INTRODUCTION

Stream ecosystems play a critical role in supporting biodiversity, biogeochemical cycles and human well-being (Aufdenkampe et al., 2011; Lynch et al., 2023; Tickner et al., 2020). Yet, these benefits can be compromised by the increasing number of anthropogenic stressors, that is, any abiotic variable that can be modified by human intervention, causing detectable changes in riverine biota or functioning (Birk et al., 2020; Reid et al., 2019; Sabater et al., 2019). Recent evidence has shown that the simultaneous alteration of multiple abiotic factors can result in cumulative ecological impacts that are difficult to predict from their individual effects (Birk et al., 2020; Lemm et al., 2021; Rillig et al., 2023). However, previous studies have mainly focussed on single-stressor effects or reduced spatial scales (e.g., laboratory experiments), which limit our ability to predict the combined effects of multiple stressors on stream ecosystems (Gutiérrez-Cánovas et al., 2022; Rillig et al., 2023; Segurado et al., 2022). Therefore, there is a need to investigate realistic multiple stressor gradients to better understand how stream degradation can alter ecosystem functions and biogeochemical processes that sustain biodiversity and human welfare (Brauns et al., 2022).

Stream waters often have higher concentrations of dissolved carbon dioxide (CO_2) and methane (CH_4) than the atmosphere. This is primarily due to hydrologically mediated inputs from terrestrial sources, such as soil respiration and geochemical weathering, as well as the in-stream production and processing of organic matter through aerobic and anaerobic metabolism (Drake et al., 2018; Raymond et al., 2013; Rosentreter et al., 2021). However, recent research has suggested that human activities may also affect the concentration of greenhouse gases (GHGs) in streams due to alterations in nutrient concentrations (Yu et al., 2017; Zhang et al., 2021), dissolved oxygen (Blaszczak et al., 2023), water temperature (Yvon-Durocher et al., 2017) and riparian vegetation (Bernhardt et al., 2022). Nevertheless, it remains unclear how much of the changes in CO_2 and CH_4 concentrations can be attributed to local carbon processing versus upstream metabolism and terrestrial sources.

Both aerobic and anaerobic metabolism can increase the local production of GHGs (local processes, Figure 1). A net CO_2 production occurs locally when in-stream respiration exceeds primary production (Campeau & Del Giorgio, 2014; Rocher-Ros et al., 2020). This process can be favoured by stream nutrient enrichment and riparian degradation (Arroita et al., 2019; Cross et al., 2022). The combination of these stressors with warming and low discharge can enhance oxygen depletion and anaerobic respiration, resulting in a higher CH_4:CO_2 ratio and overall warming potential (Campeau & Del Giorgio, 2014; Gómez-Gener et al., 2020; Yvon-Durocher et al., 2011). Anthropogenic impacts can also increase local CO_2 and CH_4 concentrations through upstream and terrestrial production (distal processes, Figure 1). This occurs when catchment-scale stressors, such as agricultural intensification, diffuse pollution and waste water effluents, enhance upstream metabolism, oxygen depletion and soil respiration (Burdon et al., 2020; Drake et al., 2018; Liu et al., 2023). However, the extent to which multiple stressors alter the contribution of local and distal processes to stream GHG concentrations remains unclear.

Previous studies have improved our understanding on how local concentrations of CO_2 and CH_4 vary in space and time over the stream continuum (Aho et al., 2021; Hotchkiss et al., 2015;
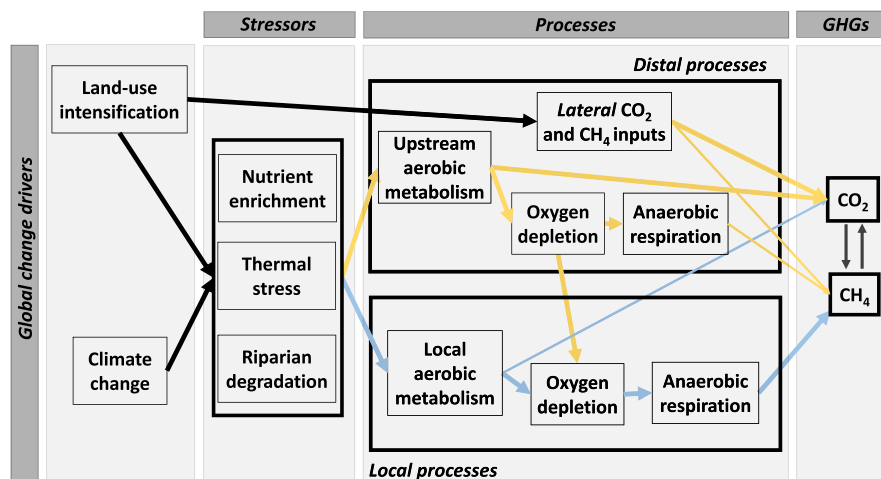

FIGURE 1 Hypothesized relationships between global change, multiple stressors and concentrations of CO_2 and CH_4 through local (blue links) and distal processes (orange links). Land-use intensification and climate change are two common global change drivers in freshwater ecosystems. Stressors are nutrient enrichment, thermal stress and riparian degradation. Local processes are represented by in situ aerobic metabolism (the balance between primary production and aerobic respiration), oxygen depletion and anaerobic respiration. Distal processes include GHGs originated by upstream aquatic aerobic metabolism, oxygen depletion and anaerobic metabolism and lateral terrestrial inputs caused by land-use intensification. We consider that stressors can also indirectly increase oxygen depletion upstream through distal processes, which can favour anaerobic respiration locally. The breadth of the arrows relating local and distal processes with GHGs indicate the strength of their expected relationship. Stream hydrology was not represented for simplicity, but it is a crucial factor for stream biogeochemistry, and can be influenced by both natural and anthropogenic variability. See Figure S1 for more details about how we tested local and distal effects in our structural equation models.

Lupon et al., 2019). However, the mechanisms that control spatial patterns of CO_2 and CH_4 over gradients of multiple stressors remain poorly understood. Although CO_2 and CH_4 concentrations are often correlated, they are typically influenced by different environmental drivers (DelVecchia et al., 2023; Yu et al., 2017; Zhang et al., 2021), reflecting different biogeochemical processes and contrasting fine-scale variability over the fluvial continuum (Campeau & Del Giorgio, 2014; Stanley et al., 2016). This occurs because of their differential water solubility and contrasting contribution of local production (Stanley et al., 2016). While CH_4 is primarily generated through local sediment methanogenesis and can easily evade or be oxidized (Stanley et al., 2016), CO_2 has a broader range of sources, with upstream production and lateral soil inputs generally playing a more important role than local metabolism (Gómez-Gener et al., 2016; Hotchkiss et al., 2015; Lupon et al., 2019). This occurs because the water solubility of CO_2 is higher than that of CH_4, leading to lower evasion rates and increased longitudinal transport of CO_2 throughout the hydrological continuum (Gómez-Gener et al., 2016; Hotchkiss et al., 2015). As such, multiple stressor impacts and controlling mechanisms can vary between these GHGs. Thus, the partial pressure of CH_4 in water (pCH_4) would be more influenced by changes in local metabolism (Campeau & Del Giorgio, 2014; Stanley et al., 2016), whereas the partial pressure of CO_2 in water (pCO_2) could respond to both local and distal processes (Hotchkiss et al., 2015; Rocher-Ros et al., 2020; Solano et al., 2023). However, these expectations have not yet been evaluated in the context of multiple stressors. A better understanding of how stressors alter these processes can reduce the uncertainty in the estimation of the stream carbon budget and aid in identifying potential feedbacks resulting from intensified global change.

Using data from an extensive survey of 50 sites across multiple stressor gradients in North Portugal, we investigated the combined effects of stressors on stream metabolism, pCO_2 and pCH_4 and the role of local and distal processes in driving these responses. To do this, we conducted simultaneous measurements of stream metabolism (gross primary production [GPP], ecosystem respiration [ER] and net ecosystem production [NEP]) from diel changes in dissolved oxygen and algal production, and stream water concentrations of CO_2 and CH_4. In our models, we considered the effects of four stressors (nutrient enrichment, oxygen depletion, thermal stress and riparian degradation) and hydrology, which is a crucial factor for stream biogeochemistry. First, we explored the effects and importance of stressors and hydrology on stream metabolism and GHG concentrations. We used model results to visualize how CO_2 and CH_4 concentrations, expressed in CO_2-equivalent concentrations, are associated with the most important stressors. Second, we hypothesize that local stream concentrations of CO_2 and CH_4 will be influenced by multiple stressor effects operating at different spatial scales (Figure 1; Figure S1). Specifically, we expect that pCO_2 will be primarily associated with distal processes, including upstream production and lateral terrestrial inputs. In contrast, we predict that pCH_4 will be controlled locally through dissolved oxygen deficit and heterotrophy, which in turn will be driven by variations in

multiple stressors and discharge (Figure 1). To test our hypothesis, we evaluated local and distal pathways linking stressors and GHGs concentrations via in-stream metabolism, with direct effects representing distal stressor controls on CO_2 and CH_4 concentrations, and indirect pathways indicating stressor effects mediated by changes in local sources (stream metabolism and dissolved oxygen; Figure 1; Figure S1).

2 | METHODS

2.1 | Study area and sampling design

The study was conducted in 50 stream sites in North Portugal. Surveyed watercourses belonged to the Ave, Cávado, Lima and Minho catchments. Climate is Atlantic with warm, dry summers (mean temperature: 11.5°C) with a mean annual precipitation around 1000 mm (Portal do Clima, http://portaldoclima.pt/en/; Trigo & DaCamara, 2000). Lithology is siliceous, dominated by granite rocks, but also including slates and schists.

Our sampling design, carried out during June–October 2020, aimed to represent realistic gradients of multiple abiotic factors that can potentially act as stressors for stream ecosystems (Brauns et al., 2022; Gutiérrez-Cánovas et al., 2022; Lourenço et al., 2023). We define a stressor as any abiotic variable that can be altered by human intervention, resulting in detectable changes in riverine biota or functioning (Birk et al., 2020; Sabater et al., 2019). To do this, our site selection covered crossed gradients of catchment size, catchment land-use cover and reach-scale riparian vegetation cover to capture wide ranges of nutrient enrichment, dissolved oxygen depletion, thermal stress, riparian canopy openness and discharge (Table S1). These crossed gradients represent a spectrum of stress intensities and combinations that simulate realistic global change scenarios for land-use intensification and climate change in the study area (Table S1), comparable to those used in manipulative experimental studies (e.g., Cross et al., 2022; Matthaei et al., 2010; Romero et al., 2018).

The study site selection encompassed a range of land-uses, from natural conifer and broadleaf forests to a variety of mixed land-use types, such as extensive agriculture (cereals, corn), pastures and diffuse urban areas. This produced a wide variation in nutrient concentrations (dissolved inorganic nitrogen ranged 0.03–4.69 mg L^{-1}) and dissolved oxygen concentration deficit (0.3–7.1 mg L^{-1}). The variability in local riparian vegetation structure and density at the reach level allowed us to capture a range of riparian canopy openness (33%–100%) (Pace et al., 2022). Additionally, variations in elevation (6–930 m a.s.l.) and local climate (July 2020's mean air temperature ranged 8.7–29.0°C) permitted us to capture a wide range of daily mean water temperatures (12.2–25.7°C). To explore whether the effect of stressors on stream metabolism and GHG production depends on stream hydrology and size, we covered wide ranges of flow velocity (0.007–0.323 m s^{-1}), stream width (1–35 m) and discharge (0.001–1.731 L s^{-1}).

2.2 | Characterization of stressor and environmental gradients

At each sampling site, we characterized indicators of nutrient enrichment, oxygen depletion, thermal stress, riparian degradation and stream hydrology (Table S1). Dissolved inorganic nitrogen (DIN) was used as a proxy of nutrient enrichment, as it represents different nitrogen compounds (nitrite, nitrate and ammonium) and showed a positive correlation with dissolved phosphate ($r_P = .56$, $p < .001$). The concentration of these solutes was determined from water using a HACH DR/2000 spectrophotometer (Hach Company, Loveland, CO, US). Water samples were collected in plastic bottles, transported cooled and frozen until analysis. We calculated mean daily dissolved oxygen deficit in the water column (DO deficit) as an indicator of oxygen depletion and hypoxic conditions that can favour anaerobic respiration (Blaszczak et al., 2023; Gómez-Gener et al., 2020). For each river reach, DO deficit was calculated as the difference between the DO saturation concentration and mean daily DO concentration. DO was recorded every 10 min during 24 h using miniDOT loggers (PME, USA). One oxygen datalogger was placed and fixed to the riverbed of each sampling site at 10–30 cm depth using iron bars. We estimated mean water temperature as an integrative indicator of thermal stress. We calculated mean daily temperature for each site from continuous temperature recording of 32–46 days obtained using HOBO UA-002-64 dataloggers (Onset, USA). To represent the effects of riparian degradation, we estimated riparian canopy openness, which reflects increasing human and climatic impacts on riparian vegetation structure and an increase in light inputs (Feld et al., 2018; Munné et al., 2003; Pace et al., 2021). Based on the normalized difference vegetation index (NDVI) of the riparian vegetation (from pure riparian vegetation pixels), we calculated canopy openness for each 100-m reach as 1 − NDVI values (Pace et al., 2022). As such, the resultant values indicate greater canopy openness when they are close to 1. NDVI values were obtained from Sentinel-2 orthorectified surface reflectance images (S2A MSI L2A). To cover the whole study area, we selected two clear scenes (T29TNG and T29TNF tiles) from June 2020 (Copernicus Open Access Hub user interface, developed by the European Space Agency for Earth observation). To characterize hydrology and control for stream size, we used discharge—a crucial hydrological indicator that responds to variations in catchment size, climate, hydromorphology and land-use alterations (Bussi et al., 2018; Vicente-Serrano et al., 2019). Discharge was calculated as the product of mean width, mean depth and mean current velocity at the reach level. These variables were calculated across five transects over a 100-m reach. For each transect, we recorded at least five measures of depth and current velocity using a flow meter with a graduated stick (Electromagnetic Flow Meter-Model 801, Valeport, UK). We also characterized segment slope for each location from a digital elevation model. More details on stressor and environmental characterization are available in Lourenço et al. (2023).

2.3 | Stream metabolism

We used two approaches to characterize stream metabolism, including estimates of daily ecosystem GPP and respiration (ER) and estimates of algal production covering a longer time frame (~5 weeks).

For each study site, we used continuous dissolved oxygen measurements (see above) to estimate GPP and ER with the open-channel single-station method (Odum, 1956). This mass-balance-based approach fits modelled DO to observed DO to estimate ER, GPP and a standardized rate coefficient for gas exchange (k_{600}), using maximum likelihood fit in the *streamMetabolizer* R package (Appling et al., 2018). To limit the range of model solutions and reduce problems related to equifinality (Appling et al., 2018), we constrained model solutions by using a prior value for k_{600} at 20°C (m day^{-1}) for each study site. k_{600} was estimated from segment slope (s, m m^{-1}) and flow velocity (v, m s^{-1}) following eq. (3) in Raymond et al. (2012):

$$k_{600} = 1162\, s^{0.77}\, v^{0.85}. \tag{1}$$

Net ecosystem production was calculated as the difference between GPP and ER. We transformed oxygen-based rates to carbon metabolic rates (mmol C m^{-2} day^{-1}) using a CO_2:O_2 ratio of 138:106 (Torgersen & Branco, 2007). To evaluate the sensitivity of the metabolic estimates to the quantification of the gas exchange coefficient, we explored the relationship between k_{600} and ER (Figure S1) and found no significant relationship ($r_P = .236$, $p = .098$). To assess the performance of metabolic estimations, we inspected plots comparing observed and modelled diel DO values (Figures S3 and S4) and calculated mean absolute error (MAE) of the modelled DO values (Table S2; Figure S5). As a result, we discarded the metabolism rates of four sample sites due to non-plausible values (GPP < 0) or because they show very high MAE (i.e., reflecting inaccurate estimation of metabolic rates). We retained 92% of our initial data set ($n = 46$). See Table S2 for more details.

To calculate algal production (mg Chl *a* m^{-2} day^{-1}), we measured the accumulation of chlorophyll-*a* on grazing-protected colonization tiles (10 × 10 cm unglazed tiles) over a period of ~35 days (range: 32–46 days). We used grazing-protected tiles to avoid noise caused by grazing. At each sampling site, we deployed three concrete bricks including one attached tile, which was surrounded by petroleum jelly to prevent grazing by crawling grazers. During the recovery day, we brushed tiles and preserved the algal material at −20°C under dark conditions. We then determined chlorophyll-*a* concentrations by spectrophotometry, following extraction in 90% acetone (Jeffrey & Humphrey, 1975). Algal production rates were calculated as the chlorophyll-*a* concentration per unit of surface divided by the number of colonization days.

2.4 | GHG concentrations and emissions

We measured the partial pressure of CO_2 and CH_4 in water with the headspace method. Using 60-mL polypropylene syringes, we collected three samples of 30 mL of stream water 10 cm below

surface, plus another 30 mL of ambient air. This procedure ensured an equal ratio of ambient air and water. Next, each syringe was vigorously shaken for 1 min to ensure an adequate gas equilibration, followed by 10 min of immersion in stream water to maintain constant equilibrium temperature. The water temperature was recorded using a multiparameter probe (Multi 3630 IDS Set KS2, WTW, Germany). From each equilibrated sample, we injected 20 mL of air into a 12-mL glass vials equipped with crimped rubber stoppers (Exetainers, Labco, UK) until analysis. The concentrations of CO_2 and CH_4 in water and air samples were analysed on an Agilent 7820A gas chromatograph equipped with a flame ionization detector. Aqueous CO_2 and CH_4 concentrations at field conditions were determined from measured headspace gas volume fractions and concentrations, based on the barometric pressure at the sampling site, field water temperature, laboratory equilibration temperature and the appropriate Henry's law constants (Sander, 2015). We collected GHG samples and measured DO data for metabolism in coincident days. We also calculated the molar ratio of CH_4 and CO_2 (CH_4:CO_2; Gómez-Gener et al., 2020; Stanley et al., 2016) and the proportion of CO_2-equivalent pCH_4 relative to the total sum of CO_2-equivalent pCH_4 and pCO_2. In this latter estimation, we assumed that CH_4 had 28 times more warming potential than CO_2 over a 100-years horizon (IPCC, 2014). We used CO_2-equivalent units to better represent the contribution of CH_4 to the overall global warming potential of dissolved gases. However, it is important to note that a fraction of these gas concentrations will not evade stream water because of potential CH_4 oxidation (Stanley et al., 2016) and photosynthetic re-assimilation of CO_2 (Solano et al., 2023).

Based on pCO_2 and pCH_4, and using Fick's first law of gas diffusion, we determined GHG fluxes across the water-air interface (F_{CO_2} and F_{CH_4}; mmol m^{-2} day^{-1}):

$$F_{CO_2} = k_{CO_2} K_{hCO_2} \Delta p_{CO_2}, \quad (2)$$

$$F_{CH_4} = k_{CH_4} K_{hCH_4} \Delta p_{CH_4}, \quad (3)$$

where k_{CO_2} and k_{CH_4} (m day^{-1}) are the specific gas transfer velocity for CO_2 and CH_4, k_{hCO_2} and k_{hCH_4} (mmol μatm^{-1} m^{-3}) are the Henry's constants for CO_2 and CH_4 adjusted for salinity and temperature (Millero, 1995; Weiss, 1974), and Δp_{CO_2} and Δp_{CH_4} are the differences between surface water and air partial pressures of CO_2 and CH_4. We assumed constant ambient air concentrations for CO_2 (pCO_2=413 ppm) and CH_4 (pCH_4=1.8 ppm) from The Global Greenhouse Gas Reference Network (NOAA, 2020). Positive flux values represent gas evasion from the water to the atmosphere and negative values indicate gas uptake from the atmosphere to the water. k_{CO_2} and k_{CH_4} values were calculated as:

$$k_{CO_2} = k_{600} \left(\frac{S_{CO_2}}{600} \right)^{-\frac{2}{3}}, \quad (4)$$

$$k_{CH_4} = k_{600} \left(\frac{S_{C_{CH_4}}}{600} \right)^{-\frac{2}{3}}, \quad (5)$$

where S_C (dimensionless) is the Schmidt number of CO_2 and CH_4 at the water temperature (Wanninkhof, 1992).

2.5 | Data analysis

Initially, we used Pearson's correlations to understand associations between primary production (GPP and algal production), ER and heterotrophy (NEP), and between concentrations and fluxes of GHG forms. We also determined correlations between DO deficit, heterotrophy and GHGs. To explore multiple stressor effects on stream metabolism, GHG concentrations and the molar ratio CH_4:CO_2, we used linear regression models and a multi-model inference approach (Burnham & Anderson, 2002). All models included DIN, water temperature, riparian canopy openness and discharge as predictors. Models predicting responses of pCH_4 and the molar ratio CH_4:CO_2 also included DO deficit as a predictor because high DO deficit values are associated with hypoxic conditions, anaerobic metabolism and methanogenesis (Blaszczak et al., 2023; Gómez-Gener et al., 2020; Stanley et al., 2016). We conducted multi-model inference through a two-step process. First, using Akaike information criterion for small sample sizes (AICc), we evaluated whether global models for each response variable should include a pairwise interactive term besides the additive stressor terms. We retained a model with an interaction if it showed the minimum AICc value and it differed in more than two AICc units respect to the additive model. We thus fitted four models, one containing only additive terms and the other three also including a pairwise stressor interaction each. A recent synthesis revealed that nutrient enrichment is an overarching stressor for riverine ecosystems, which can interact with light, thermal and hydrological stressors across spatial scales (Birk et al., 2020). Thus, we tested three related interactive terms where nutrient enrichment effects can be modulated by riparian degradation (DIN×canopy openness), thermal stress (DIN×water temperature) or hydrology (DIN×discharge). Second, based on the selected global model for each response variable, we quantified stressor coefficients, statistical support and importance, using the function *dredge*() from the *MuMIn* R package (Bartoń, 2016). This function produces the models for all potential combinations of predictors included in the global model. Based on their AICc values, we ranked the alternative models for each response variable and retained those with an AICc difference ≤7 with respect to the highest-ranking model. We also derived the explained variance (R^2) and Akaike weights to determine the explanatory power and the relative likelihood of each model (statistical support), respectively. For each response variable, based on model's Akaike weights, we obtained the mean-weighted partitioned variance for each predictor (Hoffman & Schadt, 2016). To visualize the overall response of these models and using model's Akaike weights, we calculated a weighted-average of their standardized regression coefficients and predictions across the retained models (ΔAICc ≤7).

Using the fitted values from the selected models for CO_2 and CH_4, we explored how CO_2-equivalent concentration could change within the observed ranges of nutrient enrichment (DIN: 0.25–5 mg L^{-1}) and

oxygen depletion (DO deficit: 0.25–7.mg L^{-1}). To do so, we considered that CH_4 had 28 times the warming potential of CO_2 over a 100-year horizon (IPCC, 2014). We use CO_2-equivalent units to better represent the warming potential of CH_4 relative to CO_2 for different stressor levels, given that these GHGs could require different mitigation strategies. We focused on nutrient enrichment and oxygen depletion because they were the two most important predictors of CO_2 and CH_4 in our linear regression models, and because of their importance in management policies and restoration actions in a multiple stressor context (Birk et al., 2020; Feld et al., 2018; Spears et al., 2021).

To quantify the role of local and distal processes in mediating multiple stressor impacts on GHG concentrations (Figure S1), we used a structural equation modelling (SEM) approach (*piecewiseSEM* package; Lefcheck, 2016). SEM is a causal inference tool that can be used to examine complex relationships in ecosystems because several influences and responses are analysed simultaneously (Grace et al., 2010). Therefore, SEM allowed us to determine to which degree CO_2 and CH_4 responses to multiple stressors (DIN, canopy openness and water temperature) and hydrology (discharge) were mediated by indirect changes in metabolism (NEP) and DO deficit (only for CH_4) occurring locally or by catchment-level processes. Local effects were modelled as the stressor and hydrological effects directly influencing NEP and those arising from NEP towards DO deficit or GHGs. Distal effects were represented by the direct associations between stressors and DO deficit or GHGs, which indicate that CO_2 and CH_4 are mainly produced elsewhere in the upstream section or the terrestrial environment. We built two SEMs regarding our hypothesized controlling mechanisms for CO_2 and CH_4 concentrations (see Figure S1 for more details about the full SEM structure):

a. A SEM explaining CO_2 concentrations that included two coupled models (Figure S1a): the first relating NEP with multiple stressors and the second predicting pCO_2 changes from NEP and multiple stressors;

b. A SEM explaining CH_4 concentrations that included three coupled models (Figure S1b): one model associating multiple stressors and discharge with NEP, coupled with a model predicting DO deficit from NEP, stressors and discharge and, finally, another model predicting pCH_4 from DO deficit, stressors and discharge.

The overall model fit was assessed using Fisher's *C*-test, in which small and non-significant values (*p*-value > .05) indicate a good fit of the model (Shipley, 2013). Following these criteria, we removed stressor interactions and stressors links with non-significant *p*-values and low predictive capacity to increase model performance (i.e., reducing *C*-test value and increasing *p*-value). We focussed on the observations for which complete data were available (*n* = 46). For each stressor, we quantified the contribution of the effects of local and distal processes on GHGs by summing their standardized effect sizes across the SEM structure without consideration of the direction of the relationship (absolute standardized effect size). For indirect stressor–GHG relationships, we multiplied stressor effect

sizes by the effect size of the variable mediating their effects on each GHG (i.e., NEP and/or DO deficit).

For all models, some variables were log-, logit- or squared-root-transformed to reduce distribution skewness and all variables were *z*-standardized (mean = 0, SD = 1) to allow the comparison of model coefficients. In all models, predictor combinations showed variance inflation factors <3, suggesting an acceptable degree of collinearity (Zuur et al., 2009). Model residuals were visually assessed to verify linear model assumptions (Zuur et al., 2009). All statistical analyses were performed using the R statistical software version 3.4.1 (R Development Core Team, 2011). The data and R code required to reproduce our results are available at the GitHub (https://github.com/tanogc/multiple_stressors_ghg/) and Zenodo repositories (Gutiérrez-Cánovas et al., 2024).

3 | RESULTS

3.1 | Spatial variation of stream metabolism and GHG concentrations and emissions

The stream reaches in our study showed a median GPP of 22.2 mmol C m^{-2} day^{-1} and a median algal production of 0.072 mg Chl *a* m^{-2} day^{-1} (Figure S6). However, there was considerable variation in these values, ranging from 0.1 to 226.7 mmol C m^{-2} day^{-1} for GPP and from 0.002 to 0.790 mg Chl *a* m^{-2} day^{-1} for algal production. In contrast, the streams exhibited generally high rates of respiration (ER), with a median of 76.6 mmol C m^{-2} day^{-1}, ranging from 6.5 to 613.9 mmol C m^{-2} day^{-1}. Consequently, all the streams were heterotrophic, with a median NEP of −56.1 mmol C m^{-2} day^{-1} and a range of −474.9 to −6.4 mmol C m^{-2} day^{-1}. ER was positively correlated with both GPP (r_P = .85, *p* < .001; Figure 2a) and algal production (r_P = .53, *p* < .001). Likewise, increasing primary production was associated with greater heterotrophy, as indicated by the negative relationship between NEP and GPP (r_P = −.68, *p* < .001) and between NEP and algal production (r_P = −.51, *p* < .001). Increasing heterotrophy was also linked to a greater DO deficit (r_P = −.41, *p* < .01; Figure 2b).

Surface waters were generally oversaturated with respect to the atmosphere for both pCO_2 and pCH_4, resulting in net emissions of CO_2 and CH_4 (Figure 2c,d; Figure S6). Specifically, the median pCO_2 and pCH_4 concentrations were 1064 and 4.3 µatm, respectively, and the median CO_2 and CH_4 emissions were 708 mmol CO_2 m^{-2} day^{-1} and 1.8 mmol CH_4 m^{-2} day^{-1}, respectively. Only two sites were found to be CO_2 sinks (yellow dots in Figure 2c). The mean of the molar ratio CH_4:CO_2 was 0.014, with a range of 0.002–0.041. Similarly, the contribution of CH_4 to the total CO_2-equivalent concentration was highly variable across the study area, with a median of 12.9% and a range of 2.2%–29.6%. Streams with high GHG concentrations tended to have greater emissions, as indicated by the positive correlation between concentrations and emissions for both CO_2 (r_P = .67, *p* < .001; Figure 2c) and CH_4 (r_P = .76, *p* < .001; Figure 2d). Higher concentrations of CO_2 (r_P = .74, *p* < .001) and CH_4 (r_P = .60, *p* < .001) were associated with a greater DO deficit. The positive

FIGURE 2 Relationships between gross primary production (GPP) and ecosystem respiration (ER; a) and between net ecosystem production (NEP) and the deficit of dissolved oxygen concentration (DO deficit; b). The relationships between GHG concentrations and fluxes are also shown (c, d). Dashed lines in panel a indicate the 1:1 slope, whereas dashed lines in panel c represents zero CO_2 emissions. Negative CO_2 fluxes are indicated in yellow colour. GHG concentrations in water were obtained from direct measurements (headspace method). GHG fluxes were estimated from concentrations and gas exchange coefficients. Pearson correlation and significance levels (**$p < .01$; ***$p < .001$) are displayed for each relationship.
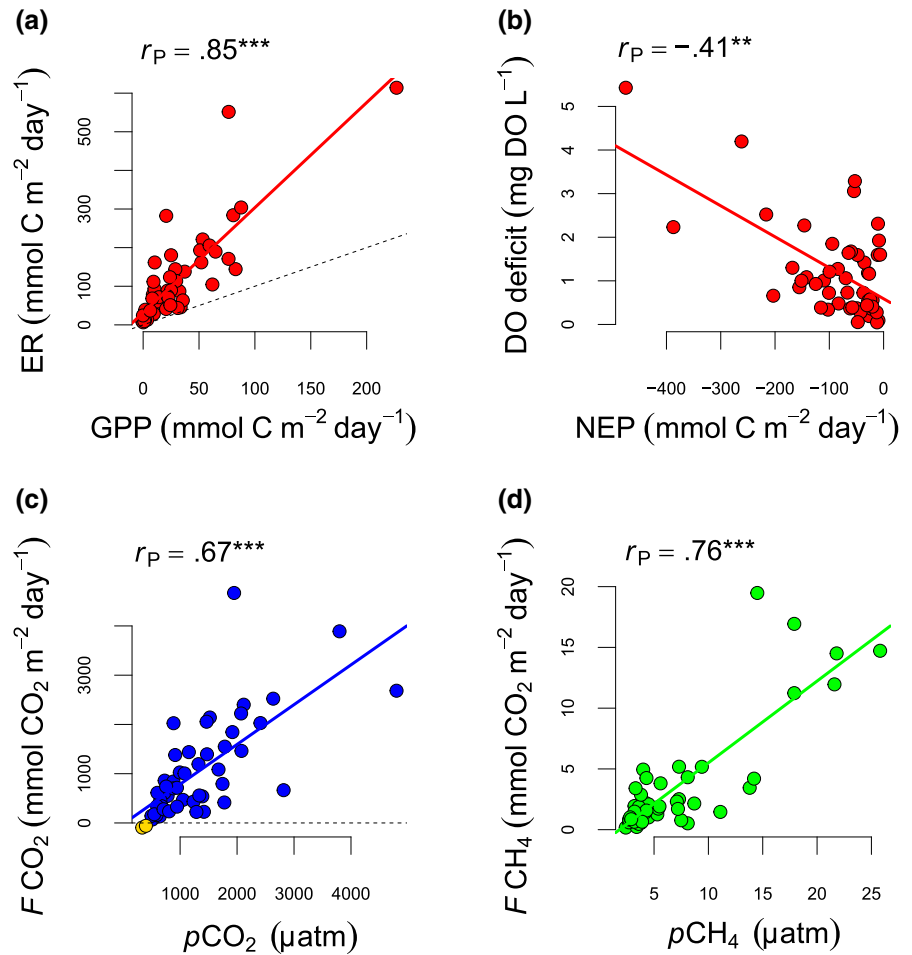
correlation between pCO_2 and pCH_4 ($r_P = .50$, $p < .001$) suggests that they shared similar spatial pattern and controls. The median of the estimated k_{600} was 16.1 m day^{-1}, but ranged from 3.2 to 56.0 m day^{-1} (Figure S7).

3.2 | Multiple stressor effects on river metabolism and GHGs

Our results suggest that stressor effects on river metabolism tend to be additive. GPP was positively associated with discharge and temperature (Figure 3a). In addition, DIN and discharge had a positive effect on algal production and respiration (Figure 3b,c), but a negative effect on NEP (Figure 3d). Discharge was the most important predictor of GPP ($r^2 = 45.3\%$; Figure 3a) followed by temperature ($r^2 = 16.2\%$; Figure 3a), whereas both DIN and discharge were the most important predictors of ER ($r^2 = 45.1\%$; $r^2 = 15.7$, respectively; Figure 3b), NEP ($r^2 = 29.1\%$; $r^2 = 22.2$; Figure 3c) and algal production ($r^2 = 26.9\%$; $r^2 = 18.9$; Figure 3d). Riparian canopy openness had a lower explanatory power and exhibited individual effects that tended to overlap with zero.

CO$_2$ concentrations showed a positive relationship with DIN but declined with discharge (Figure 4a). Both DIN and discharge were the most important predictors of pCO_2 (DIN $r^2 = 29.0\%$; discharge

$r^2 = 21.4\%$). CH$_4$ concentrations increased with DO deficit, water temperature and discharge (Figure 4b). DO deficit was the best pCH_4 predictor ($r^2 = 30.7\%$), followed by water temperature ($r^2 = 19.8\%$) and discharge ($r^2 = 2.8\%$). The effects of DIN on pCH_4 were partly modulated by water temperature ($r^2 = 7.3\%$), showing an opposing-type interaction.

Our pCO_2 and pCH_4 models showed that nutrient enrichment and oxygen depletion can jointly increase the overall amount of stream CO_2-equivalent concentrations, with a nonlinear increase of CH_4 concentration as oxygen depletion grows (Figure 5). We found that at oligotrophic (DIN = 0.25 mg L^{-1}) and oxic (DO deficit = 0.25 mg L^{-1}) conditions, the baseline CO_2-equivalent concentration was 959 μatm. However, when nutrient enrichment and oxygen depletion reach high values (DIN = 5 mg L^{-1}, DO deficit = 7 mg L^{-1}), the CO_2-equivalent concentration can increase up to 2225 μatm (132% increase). The proportion of CH_4 in the total amount of CO_2-equivalent concentration increased with oxygen depletion from a mean of 4.4% at oxic conditions to a mean of 20.5% at high oxygen depletion. Remarkably, even at oxic conditions, high nutrient enrichment can double CO_2-equivalent concentration (1977 μatm, 106% increase) compared with baseline conditions.

Our models also showed that the molar ratio CH_4:CO_2 increased with discharge and water temperature (Figure S8), which were the most important predictors (discharge $r^2 = 22.5$; water temperature
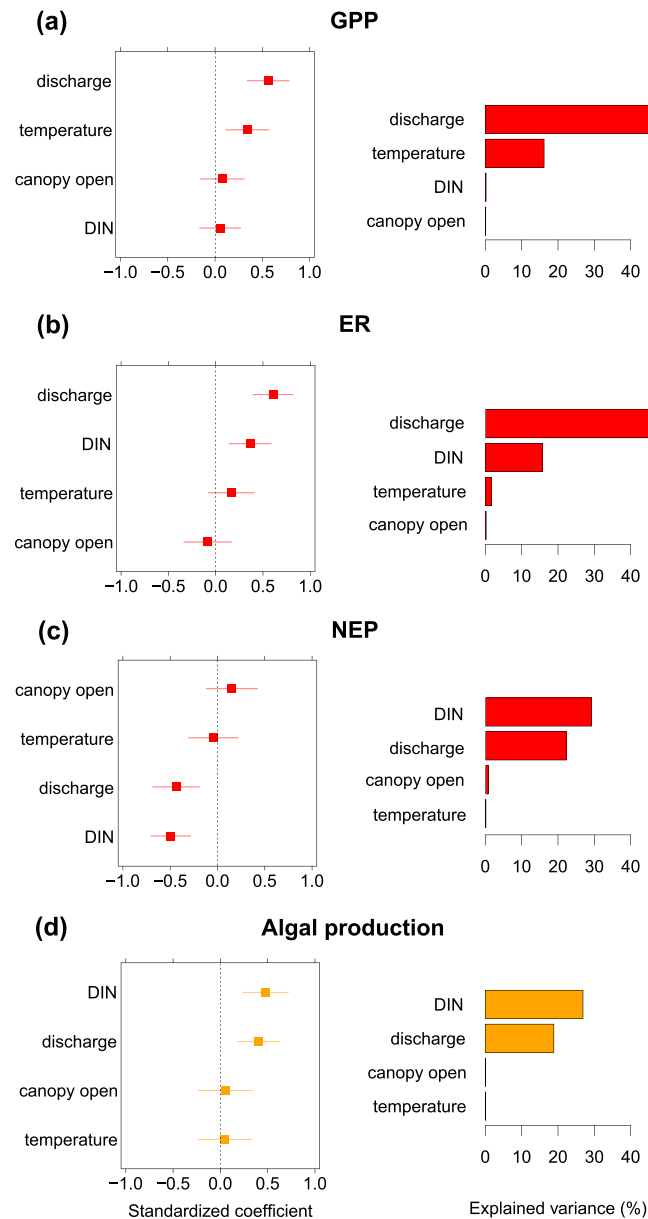
FIGURE 3 Standardized model coefficients and mean explained variance of multiple stressors and their interactions for gross primary production (GPP; a), ecosystem respiration (ER; b), net ecosystem production (NEP; c) ($n = 46$) and algal production (d) ($n = 50$). Canopy open, riparian canopy openness; DIN, concentration of dissolved inorganic nitrogen in stream water; temperature, stream water temperature.

$r^2 = 19.7\%$). Water temperature effects were modulated by DIN and followed an opposing-type interaction, which explained part of the variance ($r^2 = 12.2\%$).

3.3 | The role of local and distal processes in mediating multiple stressor impacts on GHGs

Structural equation modelling results showed that only DIN (standardized effect size, SES $= 0.475$, $p = .006$) and discharge (SES $= -0.615$,

$p < .001$) had significant direct effects on pCO_2 (Figure 6a). The indirect links between stressors and pCO_2 via NEP were not statistically supported (SES $= -0.224$, $p = .156$). Effects of DIN and discharge indicated a greater contribution of distal processes compared with local processes on pCO_2 (Figure 6b).

In contrast, pCH_4 SEM revealed that the effects of DIN and discharge on DO deficit and pCH_4 were significantly mediated by NEP (Figure 6c). Specifically, NEP was negatively related to DO deficit (SES $= -0.540$, $p < .001$), whereas DO deficit had in turn a positive effect on pCH_4 (SES $= 0.663$, $p < .001$). In addition, water temperature had a direct positive effect on pCH_4 (SES $= 0.399$, $p = .006$), but their indirect effects via NEP and DO deficit were not statistically supported. DIN effects reflected a greater contribution of local processes to pCH_4, whereas temperature and discharge effects indicated a greater influence of distal processes (Figure 6d).

4 | DISCUSSION

We demonstrate that multiple stressors play an important role in regulating CO_2 and CH_4 concentrations in streams through biogeochemical processes occurring at different spatial scales. We found that changes in pCO_2 were primarily driven by direct stressor effects reflecting upstream and lateral inputs rather than changes in local metabolism. In contrast, pCH_4 was influenced by both local metabolism and direct stressor effects that reflect distal processes. Collectively, our results underscore the importance of considering multiple stressors and both local and distal processes that influence GHG production to better understand how global change can alter GHG concentrations and fluxes in streams.

4.1 | Multiple stressor effects on GHGs

Our models revealed that nutrient enrichment and discharge, for pCO_2, and oxygen depletion and temperature, for pCH_4, were the most important drivers of GHG concentrations. These findings are consistent with recent research indicating that intensified land use and the resulting stream pollution can amplify stream metabolism (Ardón et al., 2021; Brauns et al., 2022) and GHG concentrations and fluxes (DelVecchia et al., 2023; Yu et al., 2017; Zhang et al., 2021). However, our study provides novel insights into how combined stressor effects alter both stream metabolism and GHGs. Specifically, we found that nutrient enrichment was more strongly associated with pCO_2 than with pCH_4. This pattern is probably reflecting the stronger positive effects of nutrients on aerobic respiration and heterotrophy (Cross et al., 2022). In contrast, pCH_4 was primary driven by oxygen depletion and temperature, indicating its strong dependence on conditions favourable for methanogenesis (Stanley et al., 2016). As such, our results show that temperature had a positive effect on CH_4, and the molar ratio CH_4:CO_2 (Yvon-Durocher et al., 2011, 2014), but that the effects of temperature can be modulated by an opposing interaction with DIN. Manipulative

FIGURE 4 Standardized coefficients and mean explained variance of multiple stressors and their interactions for CO_2 (a) and CH_4 (b) concentrations ($n = 50$). Canopy open, riparian canopy openness; DIN, concentration of dissolved inorganic nitrogen in stream water; temperature/temp., stream water temperature.
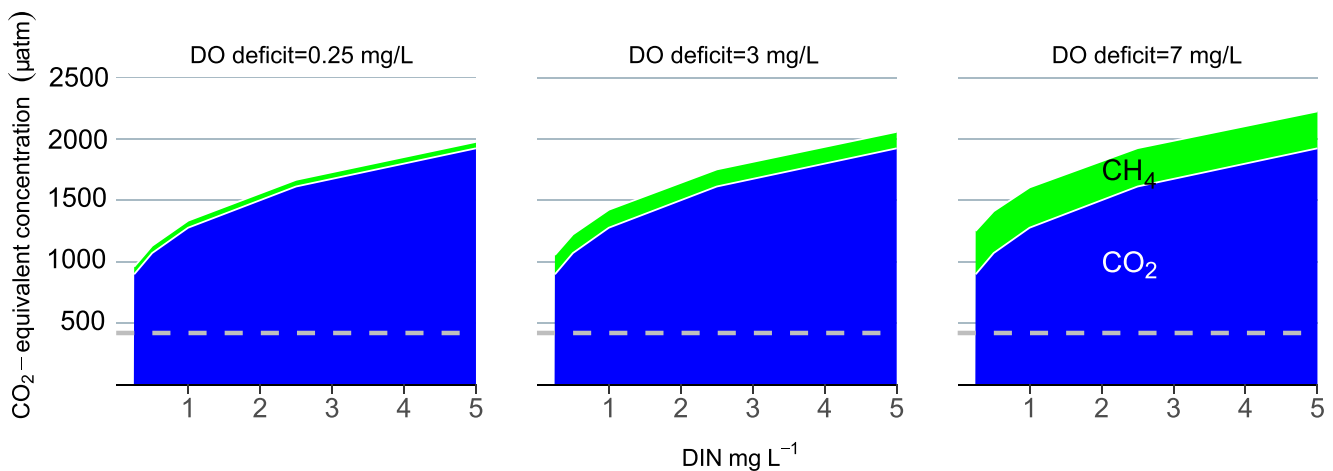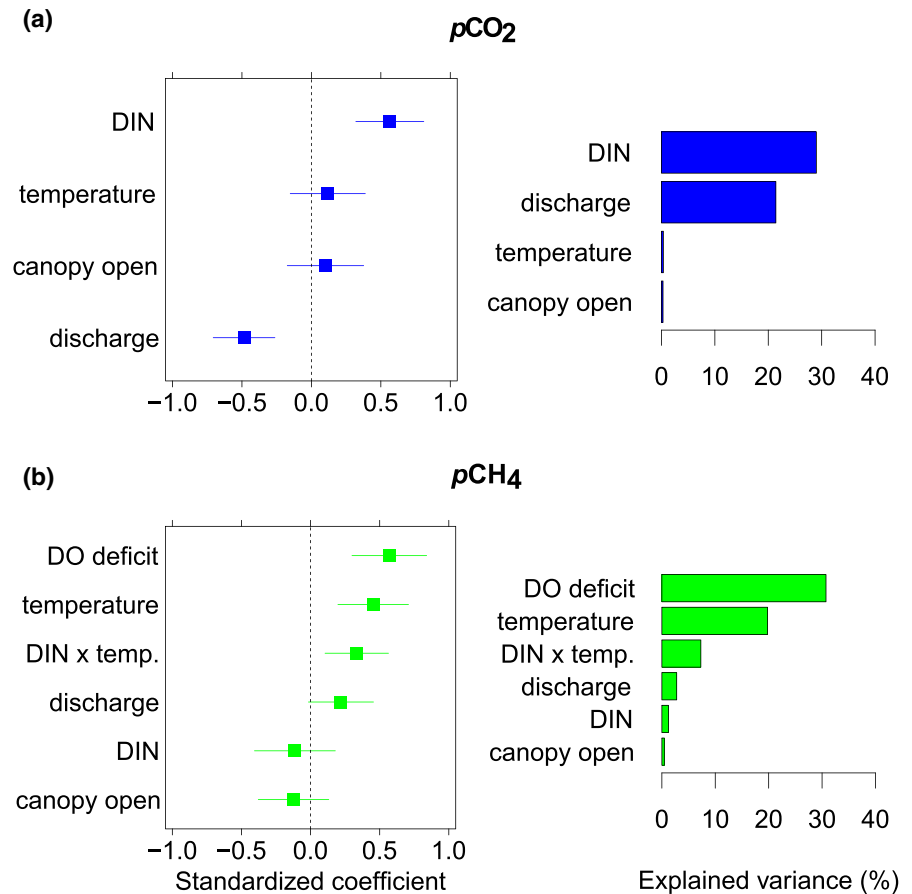

FIGURE 5 Fitted values showing the response of CO_2 and CH_4, expressed in CO_2-equivalent concentration, to different intensities of nutrient enrichment (DIN concentration) and oxygen depletion (DO deficit). Grey dashed line represents the global mean atmospheric concentration of CO_2 (413 µatm).

studies have found that nutrient enrichment and warming can jointly increase stream heterotrophy (Cross et al., 2022), but our models show a low temperature dependence of respiration. Nonetheless, we found a positive relationship between primary production and heterotrophy that might partly reflect increases in nutrient enrichment.

Consistent with the previous research (Liu & Raymond, 2018), we found that discharge was negatively associated with pCO_2, likely reflecting increased evasion rates due to more turbulent flow under

higher discharge. In contrast, our results suggest that discharge may enhance pCH_4, possibly by promoting primary production, biomass accumulation and subsequent oxygen depletion in the most productive areas (Stevenson, 1996). In addition, the positive relationship between discharge and CH_4 might reflect direct hydrological import of this gas from distal sources. These findings differ from previous studies reporting increased CH_4 concentrations during low flows as a result of prolonged residence times and consequent oxygen
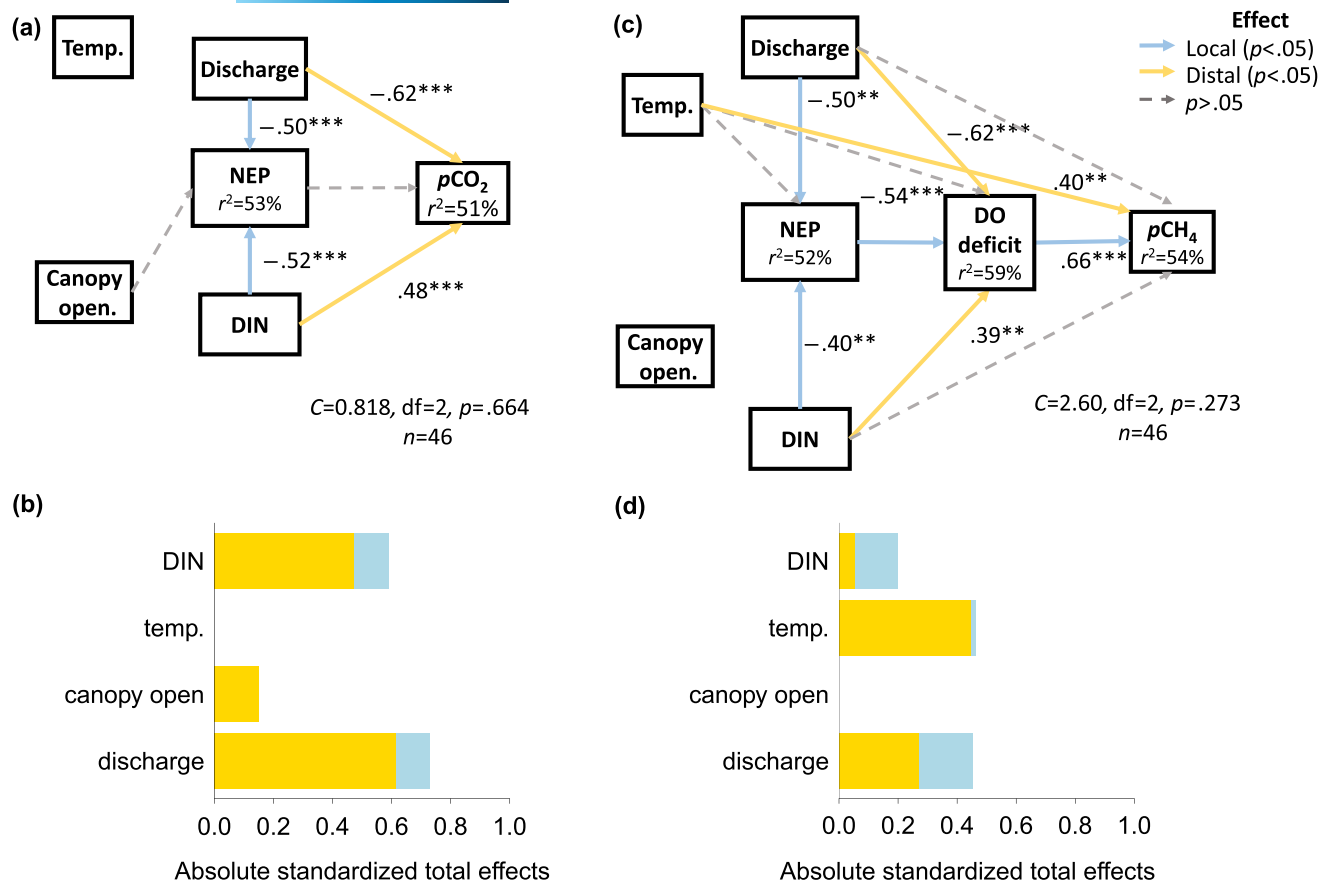
FIGURE 6 Results of structural equation models (SEMs) showing the effects of local and distal processes on pCO_2 (a, b) and pCH_4 (c, d). SEMs are shown for each GHG and include net ecosystem production (NEP) as an indirect pathway to mediate stressor effects on pCO_2 (a) and pCH_4 (c). For each stressor, we also show the absolute standardized total effects of distal (orange) and local (blue) processes on pCO_2 (b) and pCH_4 (d). Numbers and signs adjacent to each line represent the standardized effect of each predictor variable. Statistically supported links are indicated by asterisks (*$p < .05$, **$p < .01$; ***$p < .001$). Statistically non-supported links are indicated with dashed, grey lines. r^2 represents the percentage of explained variance. Canopy open, riparian canopy openness; DIN, concentration of dissolved inorganic nitrogen in stream water; DO deficit, deficit of dissolved oxygen concentration; temp., stream water temperature.

depletion (Gómez-Gener et al., 2020; Hosen et al., 2019). Such contrasting results evidence the complex interplay between hydrology and GHGs, suggesting potential nonlinear effects of discharge on primary production and pCH_4. Furthermore, we found that riparian degradation, represented by riparian canopy openness, had a weak effect on metabolism and GHG concentrations, perhaps because the sampling time (late spring and summer) ensured sufficient light inputs even in streams with dense riparian cover. To better understand its role in explaining GHG concentrations in streams, future studies would be required to capture how seasonal variations in light and leaf inputs can influence multiple stressor effects on ecosystem functioning.

4.2 | The role of local and distal processes in GHG concentrations

Our main novelty was in demonstrating that multiple stressor effects alter GHG concentrations through biogeochemical processes occurring at different spatial scales, matching our main

hypothesis. Our predictions were generally supported given that CO_2 only responded to distal processes, but the controls of CH_4 were more complex than expected. These results suggest that multiple stressors can produce impacts throughout the whole fluvial continuum, giving rise to hotspots of GHG production and emission at heavily modified catchments (DelVecchia et al., 2023; Yu et al., 2017; Zhang et al., 2021). Coupled with its higher water solubility, the increase of pCO_2 in response to human impacts stems from a variety of upstream and lateral catchment sources. Despite that internal metabolism typically produces a limited fraction of stream CO_2 (10%–19% in Hotchkiss et al., 2015; a median of 29% in Solano et al., 2023), increased metabolism following nutrient enrichment (Ardón et al., 2021; Cross et al., 2022) can give rise to an intense hydrological export of CO_2 to downstream areas. As such, land-use intensification impacts can also contribute to increase aquatic GHG concentrations across the fluvial continuum through the export of organic carbon (Drake et al., 2018). Furthermore, oxygen depletion seems to respond to both distal and local nutrient inputs and to discharge, which can partly explain the frequently observed positive associations between pCO_2

and pCH$_4$ (DelVecchia et al., 2023). This indirect stressor effect was supported by our SEMs and revealed the importance of distal processes in controlling local pCH$_4$ via increased nutrient enrichment and oxygen depletion. In addition, our results support the idea that oxygen depletion and warming caused by distal processes can favour in-stream methanogenesis (Stanley et al., 2016; Yvon-Durocher et al., 2017). This reflects the need to consider the complex interplay of factors that regulate GHG production in streams in response to global change.

Our study offers novel insights into the impact of stressors on the mechanisms regulating GHGs in streams. Previous studies did not separate the contributions of distal and local processes to local GHG concentrations (DelVecchia et al., 2023; Yu et al., 2017; Zhang et al., 2021). However, our SEMs allowed us to better understand the different pathways and spatial scales through which stressors impact GHGs and the role of local metabolism in mediating the impacts of combined stressors. Although we found that direct stressor effects were relevant for both CO$_2$ and CH$_4$, previous studies overlooking the role of local metabolism in driving multiple stressor impacts on GHGs might have been missing important biogeochemical mechanisms. In addition, we built model structures that limit spurious relationships between stressors and GHGs. For example, while the previous literature commonly reports strong negative associations between dissolved oxygen and pCO$_2$ (DelVecchia et al., 2023; Yu et al., 2017; Zhang et al., 2021), these associations are likely a result of enhanced aerobic respiration rather than direct control on pCO$_2$ (Rocher-Ros et al., 2020). Similarly, relationships between reduced solutes and pCH$_4$ shown in previous studies are more likely coincidental than explanatory (e.g., Yu et al., 2017; Zhang et al., 2021), whereas their main driver is oxygen depletion.

4.3 | Management implications

Our study highlights the potential co-benefits of policies aimed at reducing the number and intensity of stressors in order to protect river health and mitigate climate change. Our results suggest that avoiding specific stressor levels and combinations is crucial to maintain near-natural GHG concentrations. Specifically, our findings highlight that addressing nutrient pollution should be prioritized to reduce anthropogenically induced fluxes of GHG from streams. This is because nutrient enrichment was found to play a key role in influencing metabolic rates and pCO$_2$, and because most of the observed warming potential in streams, including our study sites, can be attributed to this gas (Campeau & Del Giorgio, 2014; Stanley et al., 2016). In addition, the abatement of nutrient pollution can further help to reduce GHGs concentrations by limiting the risk of eutrophication and oxygen depletion. Avoiding eutrophic and hypoxic conditions is also important for preventing CH$_4$ emissions in the context of increasing water temperatures (Kaushal et al., 2010; Kelleher et al., 2021), as these conditions will jointly exacerbate GHG emissions from polluted streams (Rosentreter et al., 2021; Yvon-Durocher et al., 2017).

The strong influence of distal processes on CO$_2$ production reinforces the need for adopting catchment-scale management perspectives to preserve stream health and avoid excess GHG production. Both diffuse and point-source pollution can increase stream metabolism and pCO$_2$ throughout the fluvial continuum. Given the global length of stream networks (Allen & Pavelsky, 2018) and their increasing degradation by multiple stressors (Schinegger et al., 2016), there is an urgent need to consider anthropogenically induced GHG concentrations in freshwaters as an important threat to climatic stability. Potential solutions include improving waste water treatment (Pereda et al., 2020), promoting soil nutrient retention (Wagg et al., 2014) and using mitigation actions that remove nutrient excess, such as riparian restoration (Feld et al., 2018). Future research should address the most effective measures and scales at which these actions should take place to limit GHG fluxes from streams.

5 | CONCLUSIONS

In conclusion, our study demonstrates the capacity of combined stressors to alter GHG concentrations in streams. Our results offer valuable insights into the processes mediating the impacts of global change on stream carbon dynamics and highlight the importance of considering multiple stressors and their differential effects at local and distal scales in predicting the response of stream ecosystems to environmental change. By identifying the most influential stressors on GHG concentrations, including nutrient enrichment, oxygen depletion and thermal stress, our study provides a new perspective for quantifying stream carbon budgets and identifying potential mitigation actions to maintain climatic stability. Our approach, which includes realistic stressor levels and combinations, can help to predict how stream ecosystem functioning and associated benefits may change in the face of ongoing global change.

AUTHOR CONTRIBUTIONS

Cayetano Gutiérrez-Cánovas: Conceptualization; data curation; formal analysis; investigation; methodology; validation; visualization; writing – original draft; writing – review and editing. **Daniel von Schiller:** Conceptualization; data curation; formal analysis; investigation; methodology; resources; supervision; validation; writing – review and editing. **Giorgio Pace:** Data curation; investigation; methodology; writing – review and editing. **Lluís Gómez-Gener:** Data curation; formal analysis; methodology; validation; writing – review and editing. **Cláudia Pascoal:** Conceptualization; funding acquisition; investigation; project administration; resources; supervision; writing – review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo at https://zenodo.org/records/10932999 and the model code is available at https://github.com/tanogc/multiple_stressors_ghg/.

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