

Elevated in-stream CO₂ concentration stimulates net-N₂O production from global fluvial ecosystems

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ABSTRACT

Riverine CO₂ and N₂O concentrations have risen since pre-industrial times, yet their biogeochemical interactions remain unclear. This study found a positive correlation between N₂O and CO₂ saturation in fluvial ecosystems, which was absent at high DOC:NO₃ ratios. Low DOC:NO₃ ratios and high CO₂ saturation promote chemoautotrophic nitrification, suggesting its key role in riverine net N₂O production. In vitro experiments confirmed that elevated CO₂ enhances nitrification rates, nitrifier gene abundance, and N₂O fluxes, indicating that the CO₂ fertilization effect on N₂O production is a potential climate feedback. Under this effect, current global N₂O emissions may be underestimated by 12 % (interquartile range: 8 – 15) due to unaccounted nocturnal CO₂ increases. As land use change projections suggest the conversion of natural lands into croplands and urban areas, this CO₂-driven rise in riverine N₂O emissions could increase, amplifying the global impacts of land use on riverine greenhouse gas emissions.

1. Introduction

Since pre-industrial times, carbon dioxide (CO₂) and nitrous oxide (N₂O) concentrations in riverine ecosystems have risen, increasing the contributions of these ecosystems to global greenhouse gas (GHG) emissions (Regnier et al., 2013; Yao et al., 2020). The concurrent increase in the concentrations of these two gases can be associated with the fact that they may have similar responses to land use and hydrological controls (e.g., Mwanake et al., 2023a; Piatka et al., 2024). Land use changes from natural forests and wetlands to agricultural lands and human settlements boost the inflow of labile carbon and nutrients to rivers, favoring in-stream CO₂ and N₂O production (e.g., Drake et al., 2019; Regnier et al., 2013; Yao et al., 2020). At the same time, over-saturated soil water, groundwater, and wastewater sources of these gases can also be directly transported to rivers during periods of hydrological connectivity (Mwanake et al., 2022, 2023b; H. Zhang et al., 2022). However, the possibility that the positive co-variation in CO₂ and

N₂O concentration in rivers could be related to a direct causal relationship between CO₂ availability and chemo-autotrophic nitrification has not been investigated.

N₂O production via nitrification in rivers can occur during ammonium oxidation. In this step, ammonium (NH₄-N) is oxidized by ammonium oxidizing bacteria (AOB) or archaea (AOA) to hydroxylamine (NH₂OH) and subsequently to nitrite (NO₂-N). The oxidation of NH₂OH or the reduction of the NO₂-N results in the formation of N₂O (Quick et al., 2019). The rate of N₂O production from ammonium oxidation depends on the abundance of ammonium oxidizers. The activity and growth of ammonium oxidizers require ammonium, oxic conditions, and the fixation of CO₂ mainly via the Calvin-Benson-Bassham (CBB) cycle for AOB and the 3-hydroxypropionate-4-hydroxybutyrate (HPHB) cycle for AOA. Compared to the HPHB cycle, the CBB cycle is more energy intensive, requiring 33 % more energy to fix one mole of CO₂. The higher energy demand of the CBB cycle is due to the inefficiency of the ribulose 1, 5-bisphosphate

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carboxylase/oxygenase (RubisCO) enzyme in catalyzing the reaction and the low affinity of the enzyme for CO₂. Therefore, ample supplies of CO₂ are required to overcome these limitations (Robert Tabita, 1999). Furthermore, RubisCO can only use aqueous CO₂ as a substrate, not HCO₃⁻ (Cooper et al., 1969), requiring an additional energy-consuming step in which HCO₃⁻ is actively transported to the cytoplasm and catalytically converted to aqueous CO₂ by the enzyme carbonic anhydrase. As HCO₃⁻ is the most abundant form of inorganic carbon in fluvial ecosystems characterized by circumneutral (pH:~7) waters (Raymond and Hamilton, 2018), the low availability of aqueous CO₂ in these ecosystems may limit the growth of ammonium oxidizers, particularly AOB, as has been shown in wastewater treatment systems (Steuernagel et al., 2018).

Apart from NH₂OH oxidation, which is either part of the nitrification process or may occur chemically (e.g., Wei et al., 2022), N₂O may be produced by AOB via a process called nitrifier denitrification, as AOB harbors the genes and enzymes homologous to those found in denitrifiers (Casciotti and Ward, 2001, 2005). This additional mechanism for N₂O production in AOB suggests that their N₂O yields may be greater than AOA's. Elevated in-stream CO₂ concentrations may directly increase N₂O production from AOB through nitrifier denitrification as it facilitates passive CO₂ fixation via the CBB cycle, thereby freeing up excess electrons for the process. Evidence for the stimulation of nitrification and N₂O production by elevated CO₂ has been reported in laboratory experiments focused on wastewater treatment (Denecke and Liebig, 2003; Jiang et al., 2015; Steuernagel et al., 2018) but has not yet been shown in riverine ecosystems. For example, Denecke and Liebig (2003) found optimum AOB growth rates in a mixed autotrophic-heterotrophic sludge reactor when CO₂ concentrations in the medium ranged from 0.01–1.04 mmol CO₂ L⁻¹, significantly enhancing rates of nitrite/nitrate formation. At the same time, higher N₂O yields from nitrification through the growth enhancement of a pure AOB culture were found in a laboratory chemostat experiment when gaseous CO₂ was the supplied carbon source instead of HCO₃⁻ (Jiang et al., 2015). Because N₂O yields from denitrification also depend on in-stream nitrate concentrations, increased nitrate turnover through nitrification may also result in higher N₂O yields from denitrification (Cébron et al., 2005; Quick et al., 2019).

Although the theoretical evidence above suggests that N₂O production from nitrification-induced processes (ammonium oxidation, nitrifier denitrification, and coupled nitrification-incomplete denitrification) can be stimulated by increased CO₂ concentration in fluvial ecosystems, this effect may only become evident when environmental conditions favor nitrification, resulting in both NO₃ and N₂O accretion (e.g., Baulch et al., 2011; Mwanake et al., 2019). Previous fluvial research shows that the stoichiometric ratio between DOC and NO₃ may be used to identify favorable conditions for autotrophic nitrification and heterotrophic processes such as respiration and denitrification (Strauss et al., 2002; Strauss and Lamberti, 2002; Taylor and Townsend, 2010). At low DOC:NO₃ ratios of <2, heterotrophic microbes, including denitrifiers, are C-limited (Taylor and Townsend, 2010). Under these conditions, nitrifiers are better competitors for available ammonium as compared to the heterotrophic bacteria (Strauss et al., 2002; Strauss and Lamberti, 2002), leading to an accumulation of excess NO₃ due to nitrification and decreased heterotrophic NO₃ demand (Taylor and Townsend, 2010). At DOC:NO₃ ratios between 2 – 5, nitrification can still occur; however, denitrification may dominate NO₃ consumption derived from nitrification and external sources (Taylor and Townsend, 2010). These stoichiometric conditions are therefore conducive to denitrification, often resulting in net N₂O production (e.g., Chen et al., 2025; C. Wang et al., 2024). For example, Chen et al. (2025) found that in urban river segments, denitrification was responsible for the majority of N₂O emissions (60–76 %), while nitrification contributed less than 40 %. This dominance was attributed to higher NO₃ concentrations and increased abundance of N₂O-producing denitrification genes due to urbanization. A similar study in urban river-lake networks in China also revealed that

denitrification was the primary source of N₂O emissions in urban rivers, whereas nitrification dominated N₂O hotspots in lakes and agricultural rivers (C. Wang et al., 2024). In contrast, at higher DOC:NO₃ >5, the general heterotrophic microbial population becomes more N-limited than C-limited, resulting in rapid NO₃ assimilation by the heterotrophic microbes (Taylor and Townsend, 2010). This conceptual understanding was expanded by including thermodynamic constraints related to reducing redox conditions, whereby at high DOC:NO₃ ratios, the growth of nitrifiers is O₂-limited as O₂ availability declines with increasing heterotrophic microbial activity (Helton et al., 2015), favoring NO₃ and N₂O consumption as terminal electron acceptors.

Despite the potential significance of riverine net N₂O production driven by chemoautotrophic nitrification, either as a precursor to higher NO₃ concentrations or as a direct contributor to N₂O (e.g., Hama-Aziz et al., 2017; Harrison and Matson, 2003; Mwanake et al., 2019, 2024; S. Wang et al., 2024; Winnick, 2021), its relative contribution and underlying environmental controls have received less attention than heterotrophic denitrification. This is because N₂O production rates from incomplete denitrification are assumed to be much higher than from nitrification (Quick et al., 2019). Such an assumption may not always be true, considering that ammonium-derived sources rather than nitrate-derived sources were recently shown to account for up to 69 % of N₂O production from global agricultural streams (S. Wang et al., 2024). Moreover, nitrification is a net N₂O-producing process, while heterotrophic denitrification may also result in N₂O consumption through its reduction to N₂ (e.g., Aho et al., 2023; Mwanake et al., 2019; S. Wang et al., 2024).

The overall objective of this study was to determine the effect of elevated in-stream CO₂ concentrations on net-N₂O production from global fluvial ecosystems and if such a relationship is linked to enhanced nitrification rates under favorable environmental conditions for the chemoautotrophic process. We hypothesized that elevated in-stream CO₂ would favor nitrification-induced net N₂O production when the environmental DOC:NO₃ ratios are low, particularly in streams of catchments dominated by human influences due to external nitrogen inputs. However, at high DOC:NO₃ ratios, the positive CO₂ effect would be absent, as reducing redox conditions and stoichiometric disadvantages will limit nitrification and favor N₂O consumption processes.

To test these hypotheses, we employed two approaches. First, we conducted a meta-analysis on a global dataset to examine the role of catchment land use in controlling the general spatial-temporal trends of fluvial N₂O and CO₂ concentrations, and assess the hypothesized positive relationship between the two GHGs along DOC:NO₃ ratio gradients that regulate nitrification rates. The global dataset comprised 4,158 data points (from 549 sites) across 24 published studies, based on field observations of N₂O and CO₂ concentrations from five continents (Fig. S7; Mwanake et al., 2019, 2022, 2023a; Stanley et al., 2023). Second, we employed experimental approaches to elucidate the mechanisms that may explain the stimulatory effect of CO₂ oversaturation on nitrification and net N₂O production rates in streams, where background water DOC:NO₃ ratios varied from low to high due to differences in land use. Specifically, we measured in vitro potential gross nitrification rates, N₂O concentrations, net N₂O production rates, and the gene abundances of ammonium oxidizers and denitrifiers under different dissolved CO₂ treatments representing concentrations within the global range.

2. Materials and methods

2.1. Meta-data analysis

2.1.1. Data compilation

For the meta-analysis, we compiled 4158 data points (549 sites) from 24 published studies based on field observations of N₂O and CO₂ concentrations over five continents (Fig. S7). The studies must have also included simultaneous water chemistry measurements, such as NH₄-N, NO₃-N, and DOC, to be added to the final data set. We obtained many

data points ($n = 2920$) from an existing GHG concentration published data set (Stanley et al., 2023). Additional already published data ($n = 1238$), composed mainly of headwater stream sites, were included from the Mara River in Kenya and five catchments in Germany (Mwanake et al., 2019, 2022, 2023a).

2.1.2. Relationship of upstream land use/land cover on in-stream CO_2 , N_2O and DOC:NO_3 ratios in the global data

To determine how land use/land cover affects the spatio-temporal heterogeneities of global riverine CO_2 saturation, N_2O saturation, and the DOC:NO_3 ratios, we carried out a simple analysis based on the global land use map for 2019 from the HILDA+ database (Winkler et al., 2021; <https://doi.org/10.1594/PANGAEA.921846>). First, we obtained catchment boundaries for all the sites in our global data from the global hydrobasin dataset (Level 12; retrieved October 7, 2024: <https://www.hydrosheds.org/products/hydrobasins>). Second, land use percentages for each site were calculated from the HILDA+ land use map, which contains six distinct land use classes: urban, cropland, forests, pasture, unmanaged grasslands, and sparse/no vegetation. The dominant land class for each catchment ($>55\%$ of a single land use class) was then determined. Catchments that did not predominantly belong to one land use class were classified as mixed land use catchments. Comparisons of the three parameters across land use classes were based on pairwise mean comparisons using the non-parametric Wilcoxon test. All these analyses were performed in R version 4.3.2.

2.1.3. Statistical analyses linking CO_2 saturation to elevated N_2O production from nitrification

We used a linear mixed-effect model (LME) to determine the effects of CO_2 concentrations and DOC:NO_3 molar ratios, and their interactions ($\text{CO}_2 * \text{DOC:NO}_3$ ratios) on N_2O concentrations in the global dataset ("lme4" package in R version 4.3.2). In the model, the $\text{CO}_2 * \text{DOC:NO}_3$ interaction term was particularly interesting as it allowed us to test the significance of CO_2 oversaturation in increasing riverine N_2O concentrations while considering multiple DOC:NO_3 ratios where nitrification is either favored or inhibited. The CO_2 and N_2O concentrations used throughout our analyses were expressed as % saturation to correct for water temperature differences across the latitudinal gradient in the global data (Eq. (1)). This calculation was done by normalizing their actual concentrations in the water (C_{aq} in moles L^{-1}) with the stream water concentrations in equilibrium with fixed atmospheric concentrations (421 ppm for CO_2 and 0.333 ppm for N_2O) and pressure (1atm) (C_{sat} in moles L^{-1}), based on Henry's gas solubility constants calculated at specific water temperatures.

$$\text{GHG saturation (\%)} = \left(\frac{C_{aq}}{C_{sat}} \right) \times 100 \quad (1)$$

Using fixed atmospheric concentrations may result in negligible to significant uncertainties in air-water GHG dynamics due to temporal or site-to-site differences in air temperature and barometric pressures (Reichenpfader and Attermeyer, 2024). However, this uncertainty was assumed to be minimal relative to water temperature changes, which we accounted for in the saturation calculations. In addition, we also tested the above relationships with molar concentrations, yielding similar conclusions, albeit with slightly different magnitudes in the effect sizes. Data for the overall statistical analyses were used as individual measurements for every site visit and not as catchment means. To ensure the validity of the effects of CO_2 and the DOC:NO_3 ratio on N_2O , the linear mixed-effects model structure included random effects of sampling dates and sites, thereby considering the vast geographical differences in the data (Fig. S7) and their temporal coverage (2001-07-02 to 2022-04-20). Model performances were assessed based on the normality of the residuals and the conditional r^2 (encompasses the variance explained by both fixed and random effects). Forest plots were then used to visualize the standardized effect sizes of the significant ($p\text{-value} < 0.05$) fixed

effects ("sjPlot" and "ggplot2" packages in R version 4.3.2). To visualize the $\text{CO}_2 * \text{DOC:NO}_3$ interaction effects in the global data predicted from the linear mixed effect model, multiple ($n=20$) $\text{N}_2\text{O}-\text{CO}_2$ slope $\pm$ SE values were generated ("interactions" package in R version 4.3.2), and their bivariate relationships with changing DOC:NO_3 ratios analyzed. Before model construction and evaluation, N_2O , CO_2 , DOC:NO_3 ratios were always transformed using the natural logarithm to meet the normality assumption.

Multivariate path analysis from a structural equation model (SEM) was also used to unravel biogeochemical interactions amongst drivers of N_2O concentrations ("lavaan" package in R) (Mwanake et al., 2023a). In the SEM, the theoretical model consisted of several multivariate regression equations (Eqs. (2)–(7) based on relationships inferred in previous studies (Quick et al., 2019). Endogenous variables in the models, which directly influence in-stream biogeochemical N_2O production and consumption processes, included water physico-chemical variables such as DO % saturation that determines the redox conditions for biogeochemical reactions and $\text{NH}_4\text{-N}$, and $\text{NO}_3\text{-N}$ concentrations that represent substrates for both nitrification and denitrification. The exogenous variables in the models, which affect in-stream N_2O concentrations directly as carbon sources for autotrophic and heterotrophic production or indirectly by influencing the endogenous variables, were water temperature, CO_2 saturation, and DOC concentrations.

Covariance structures (indicated by the $\sim\sim$ symbol) of the exogenous variables were added to the model (Eqs. (6) and (7) to represent photosynthesis and respiratory cycles that affect in-stream DO, CO_2 , and DOC concentrations. To develop the best-fit SEM, the removal of the least essential ($p\text{-values} > 0.05$) predictor variables in the theoretical SEM was done sequentially until the model with the highest parsimony fit index (PNFI) and a root mean squared error of approximation (RMSEA) of ≤ 0.05 was reached (Schumacker and Lomax, 2010). Graphical representations of the significant relationship pathways from the best-fit models, including standardized slope parameter estimates, were done using the "semPlot" package in R version 4.3.2.

$$\begin{aligned} \text{Ln } \text{N}_2\text{O saturation} \sim & \text{DO saturation} + \text{Ln DOC} + \text{Ln NO}_3 + \text{Ln NH}_4 \\ & + \text{Ln CO}_2 \text{ saturation} + \text{Water temperature} \end{aligned} \quad (2)$$

$$\begin{aligned} \text{Ln NO}_3 \sim & \text{DO saturation} + \text{Ln NH}_4 + \text{Ln DOC} + \text{Ln CO}_2 \text{ saturation} \\ & + \text{Water temperature} \end{aligned} \quad (3)$$

$$\begin{aligned} \text{Ln NH}_4 \sim & \text{DO saturation} + \text{Ln DOC} + \text{Ln CO}_2 \text{ saturation} \\ & + \text{Water temperature} \end{aligned} \quad (4)$$

$$\text{DO saturation} \sim \text{Ln DOC} + \text{Water temperature} \quad (5)$$

$$\text{DO saturation} \sim\sim \text{Ln CO}_2 \text{ saturation} \quad (6)$$

$$\text{Ln DOC} \sim\sim \text{Ln CO}_2 \text{ saturation} \quad (7)$$

2.1.4. Monte Carlo simulations investigating the sensitivity of N_2O to CO_2 and DOC:NO_3 ratio changes

In addition to assessing the interactive effects of CO_2 saturation and DOC:NO_3 molar ratios on N_2O saturation globally, we also examined how the reported underestimation of riverine CO_2 emissions due to daytime sampling bias (Gómez-Gener et al., 2021), combined with projected future global land-use changes (e.g., forest to urban or cropland: Alexander et al., 2018; Li et al., 2019), could amplify riverine N_2O emissions. Assuming GHG saturation is proportional to flux, we first estimated the impact of the reported 27 % global average increase in riverine CO_2 emissions due to nighttime emissions by Gómez-Gener et al. (2021) on riverine N_2O emissions. Using a Monte Carlo approach for uncertainty assessment ("MASS" package in R version 4.3.2), we generated 1,000 coefficient sets from a multivariate normal distribution based on the model estimates and their covariance from the LME model

(mixed-effects model linking N_2O , CO_2 , and $\text{DOC}:\text{NO}_3$ ratios). We then predicted N_2O saturation before and after applying a 27 % increase in riverine CO_2 saturation using the LME model, expressing the difference between the two as a percentage for each sampling date and site in the global dataset. The resulting relative % N_2O changes were summarized by calculating the median and interquartile range, and visualized using a histogram.

For the second part of the analysis, which focused on quantifying how land-use changes from forest to cropland or urban areas may affect riverine N_2O saturation, we also used the global mixed-effects model described above (LME) and applied Monte Carlo simulations to estimate uncertainty ("MASS" package in R version 4.3.2). Specifically, using the observed data from catchments dominated by forests, cropland, or urban areas as reference (Fig. S1), we generated 1,000 combinations of CO_2 and $\text{DOC}:\text{NO}_3$ values based on their observed global ranges in each land use class and their correlation derived from the LME model ("MASS" package in R version 4.3.2). We then predicted N_2O saturation from the 1000 new combinations in each land use class using varying model (LME) coefficients sampled from their multivariate normal distribution ("MASS" package in R version 4.3.2). The relative percentage differences in predicted N_2O saturation resulting from land use shifts (forest to cropland and forest to urban) in the generated dataset were computed and visualized in histograms, also indicating their median and interquartile range. We also applied a 27 % correction factor to account for potential nighttime CO_2 underestimation, repeating the simulation process and generating histograms with similar summary statistics.

2.2. Sediment microcosm experiment

2.2.1. Study area and sampling strategy

Microcosm stream water and sediment incubation experiments were conducted to provide process-based evidence on the biogeochemical stimulation of riverine N_2O production by CO_2 concentrations. To achieve this objective, we focused on assessing the effect of manipulated CO_2 treatments (ambient, 5000 ppm, 10000 ppm, 20000 ppm) on gross nitrification rates, N_2O saturation, net N_2O production, and functional gene abundances of nitrification and denitrification. Eight stream sites (1-3 orders; stream size classification based on (Strahler, 1952) located in two headwater catchments in Germany (Schwingbach and Loisch) were selected for this experiment (Fig. S7). The sites included two forest and two grassland sites in the Loisch catchment and two cropland and two forest sites in the Schwingbach catchment (See Mwanake et al., 2023a for extensive site details). These sites were also part of the compiled meta-data and represented the primary land use classes in the global data (Fig. S1: forests, pastures, and croplands).

The Loisch catchment is located in the mountainous region of Bavaria in southeastern Germany, with an elevation range from 616–2643 m above sea level (coordinates in decimal degrees: 47.4700, 11.0500). The catchment mainly comprises mixed forests (92 %) on the mountain slopes, pasturelands, and drained wetlands with intensively and extensively managed grasslands (8 %) at the valley bottoms. The climate is cold and temperate (Dfb, Köppen climate classification; Kottek et al., 2006), with annual precipitation of 1,693 mm (monthly mean min: 87 mm, monthly mean max: 207 mm) (1999 – 2019) and a mean annual temperature of 3.8 °C (monthly mean min: -6.6 °C, monthly mean max: 13.1 °C) (1991 – 2021) (Climate-data.org, Link). The Schwingbach catchment is located in the central-German state of Hesse, with a much lower elevation ranging from 176 – 480 m above sea level (coordinates in decimal degrees: 50.5000, 8.6100). The catchment comprises mixed land uses with ~47 % forests, ~45 % fertilized croplands, and 8 % settlement areas (Wangari et al., 2022). The climate is warm and temperate (Cfb, Köppen climate classification; Kottek et al., 2006), with an annual rainfall of 742 mm (monthly mean min: 51 mm, monthly mean max: 72 mm) (1999 – 2019) and a mean annual temperature of 9.8 °C (monthly mean min: 1.3 °C, monthly mean max: 18.8 °C) (1991 – 2021) (Climate-data.org, Link).

2.2.2. Sediment, water, and gas sampling

To mimic close-to-natural conditions in our microcosm experiments, we sampled both stream sediments and water from the eight sites on two dates in autumn (4/10/2022 and 20/10/2022). The choice of the autumn season was based on past experience from the studied sites (See Mwanake et al., 2023a). During the autumn season, the selected sites were characterized by the lowest discharge (Mwanake et al., 2023a), i. e., conditions we assumed to be associated with the highest sediment retention times due to the low flow velocities, thus providing the best timing for sediment sampling. At every site visit, sediment samples were randomly collected from ~10 locations within a 2 m reach at ~0 – 10 cm depths. The sediment sub-samples at each site were merged and filtered in the field through a 5 mm sieve to remove large stones and plant materials. The homogenized and filtered sample was placed in acid-washed plastic containers (2 L). In addition, stream water required for incubation was sampled in acid-washed plastic containers (10 L). The sediment and water samples were transported to the laboratory at Karlsruhe Institute of Technology, Campus Alpin, within 24 hours of collection for incubation.

On-site observations at the time of sampling included measurements of in-stream water temperature (°C), electrical conductivity ($\mu\text{S cm}^{-1}$), dissolved oxygen (DO) (% saturations), and pH using a Pro DSS multiprobe (YSI Inc., USA). Water samples were also collected from the stream sites in triplicates for physico-chemical analyses. The samples were filtered on-site through polyethersulfone (PES) filters (0.45 μm pore size and pre-leached with Milli-Q water) into 30 ml acid-washed HDPE bottles and stored in the refrigerator at 4 °C before being analyzed for dissolved N and C. Dissolved inorganic nitrogen (DIN) concentrations in the samples were quantified using colorimetric methods, whereby the absorbance of the samples and standards of known concentrations (0.1, 0.5, 1, 2, 3, 4, and 5 mg L^{-1} N) were measured on a microplate spectrophotometer (Model: Epoch, BioTek Inc., USA). $\text{NO}_3\text{-N}$ concentrations were analyzed by adding Griess reagent for color formation, and the absorbance of the samples was measured at 540 nm (Patton et al., 2011). $\text{NH}_4\text{-N}$ concentrations were analyzed using the indophenol method by measuring the absorbance of the samples at 660 nm (Bolleter et al., 1961). DOC and TDN concentrations were measured simultaneously using a DOC/ TDN analyzer (Dimatoc 2000, Dimatec, Germany).

We collected triplicate gas samples at each of the eight sites using the headspace equilibration technique (Raymond et al., 1997) to quantify *in-situ* dissolved GHG concentrations. In brief, 80 ml of stream water was equilibrated with 20 ml of atmospheric air in a syringe by shaking it in stream water for two minutes. The headspace equilibrated gas samples (17 ml) were then drawn from the syringes and stored in pre-evacuated 10 ml glass vials for later analysis in the laboratory. Atmospheric air samples were also collected at every site visit to correct for background atmospheric GHG concentrations in the headspace before equilibration. GHG concentrations in the gas samples were analyzed using an SRI gas chromatograph (8610C, Germany) with an electron capture detector (ECD) for N_2O and a flame ionization detector (FID) for CO_2 concentrations.

Dissolved CO_2 and N_2O concentrations in the stream water (C_{sw}) in moles L^{-1} were calculated based on the difference between post-equilibration gas concentrations in the headspace ($C_{\text{post},h}$) in moles L^{-1} and the water ($C_{\text{post},w}$) in moles L^{-1} , with the pre-equilibration gas concentrations in the headspace ($C_{\text{pre},h}$) in moles L^{-1} to correct for the atmospheric concentrations that were initially in the headspace (Eq. (8) (Aho and Raymond, 2019). We used the ideal gas law to calculate the headspace CO_2 and N_2O concentrations in moles L^{-1} . The water-phase CO_2 and N_2O concentrations in moles L^{-1} were calculated using Henry's Law (Eq. (9)).

$$C_{\text{sw}} = C_{\text{post},w} + C_{\text{post},h} - C_{\text{pre},h} \quad (8)$$

$$C_w = P \times H_s \quad (9)$$

Where C_w represents either the actual dissolved gas concentration in the stream water in moles L^{-1} or the theoretical gas concentration in the stream water if it were in equilibrium with atmospheric concentrations in moles L^{-1} , P is the partial pressure of the gas in atm (mixing ratio in ppmv from GC $\times$ atmospheric pressure), and H_s is the Henry's solubility constant for CO_2 (Weiss, 1974) and N_2O (Weiss and Price, 1980) in moles $L^{-1} atm^{-1}$. Both concentrations were then expressed as % saturations similar to the global dataset (Eq. (1)).

2.2.3. Experimental setup

For each site, 10 g of fresh sediment and 50 ml of stream water were first added to 15 (6 for control+9 for CO_2 treatments) 140 mL glass bottles (Sigma Aldrich). 1 ml of ^{15}N - NO_3 (5000 μ moles/L, ^{15}N at 99 %) was then added to each bottle for the quantification of gross nitrification rates (GNR) using the isotopic pool dilution method (Murphy et al., 2003) and the membrane inlet mass spectrometer for measurements of $^{15}NO_3$ -N concentrations (Lin et al., 2021). The 6 control bottles for each site included 3 replicates for initial conditions before incubation (T_0) and 3 replicates to be sampled after incubation (T_1). All 6 control bottles were capped using gas-tight lids with rubber stoppers on the top to allow headspace gas sampling. The glass bottles were placed in the shaker at 250 rpm for mixing. After mixing on the shaker for 4 min, half of the bottles (3) were incubated in the dark for 45 h in a shaking water bath (rpm 120, 20 °C) to mimic sediment and stream water movements along natural ecosystems. The other half were destructively sampled for initial concentrations of water physico-chemical variables and dissolved GHG concentrations.

In addition to CO_2 production during incubation of the 3 control bottles with ambient headspace conditions, we manipulated CO_2 and O_2 headspace concentrations in separate sediment-slurry bottles before incubation (3 treatments of 3 replicates each = 9 bottles). These treatments mainly aimed to study the effect of CO_2 and O_2 concentration changes in water samples on N_2O production. The targeted CO_2 concentrations after the enrichment were aimed to be within the range of field measurements but sufficient to cause an effect on ammonium oxidizing communities during the short incubation period, i.e., within the optimal range of 0.0108 – 1.0426 mmol $CO_2 L^{-1}$ for AOB growth rates (Denecke and Liebig, 2003). For the treatment, the 9 bottles were first capped using gas-tight lids with rubber stoppers on the top. We then manipulated the headspace concentrations in the bottles by replacing the ambient air (~ 20 % O_2 and 410 ppm CO_2) in the 80ml headspace with different volumes of 20000 ppm CO_2 in a helium atmosphere. The final three headspace treatments were 5000 ppm of CO_2 and 15 % of O_2 , 10000 ppm of CO_2 and 10 % of O_2 , and 20000 ppm of CO_2 and 0 % of O_2 . The rationale for decreasing headspace O_2 while increasing CO_2 concentrations was to mimic respiration and avoid creating artificial experimental artifacts, i.e., constant O_2 at elevated CO_2 conditions. These 9 treatment bottles were incubated simultaneously in the dark and under conditions similar to the 3 (T_1) control bottles.

2.2.4. Measurement of dissolved gases

Sampling initial (T_0) and final (T_1) concentrations of dissolved gases involved taking duplicate samples from each bottle's headspace before opening the gas-tight lids. The headspace concentrations of CO_2 and N_2O were measured on a gas chromatograph (SRI, 8610C, Germany). These concentrations were then converted to dissolved concentrations in the aqueous phase using the ideal gas law and Henry's law described above for the in-situ samples and then expressed as % saturations. After gas sampling, the bottles were opened, and 0.2 ml of saturated $ZnCl_2$ solution was added to each bottle to stop the microbial activity. 30 ml of the sediment-slurry was gently transferred to pre-washed plastic beakers for the initial and final DO and pH measurements using a Pro DSS multiprobe (YSI Inc., USA).

2.2.5. Measurement of dissolved inorganic nitrogen

Initial (T_0) and final (T_1) concentrations of DIN in the slurry mixtures

were measured by transferring 10 ml of the remaining slurry to 50 ml centrifuge tubes and adding 40 ml of 1 M KCl to the slurry mixture to extract the DIN. During extraction, the KCL and the slurry mixtures were shaken for 1 h at 250 rpm. The mixtures were then allowed to decant, and the upper waters were filtered through a 0.45 μ m pore-size PES filter for later analyses of $^{15}NO_3$ -N, total NH_4 -N, and total NO_3 -N concentrations in the filtrates. From the extracts, total NH_4 -N and NO_3 -N concentrations were analyzed using the colorimetric method described above, and DOC was analyzed using a Dimatoc 2000 (Dimatec, Germany). The initial and final $^{15}NO_3$ -N in the extracts were then analyzed on the membrane inlet mass spectrometer (MIMS) using the REOX/MIMS method (Lin et al., 2021).

2.2.6. Measurement of $^{15}NO_3$ -N using the (REOX/MIMS) method

The REOX (REduction-OXidation)/MIMS method is both sensitive (~0.1 μ M) and also precise (relative standard deviation = 0.1– 4.37 %) in quantifying $^{15}NO_3$ -N concentrations in liquid samples with a wide range of concentrations (0.1– 500 μ M) and salinities (0–35 ‰) (Kana et al., 1994; Lin et al., 2021). To quantify the $^{15}NO_3$ -N in our extract samples, we transferred 15 ml of the extract to 50 ml centrifuge tubes. About 250 mg of zinc powder and 75 μ L of H_2SO_4 were added to each sample, and the centrifuge tubes were placed in the shaker (250 rpm at room temperature) for 30 min. During the mixing, the $^{15}NO_3$ -N in our samples was reduced to $^{15}NH_4$ -N by the zinc powder in the acidic medium. The liquid phase of the mixtures was then gently transferred into 12 ml borosilicate glass vials (Labco) until they brimmed, and the vials were tightly capped with gas-tight septum caps. The next step entailed oxidizing $^{15}NH_4$ -N in the samples to dissolved N_2 isotopes ($^{29}N_2$ and $^{30}N_2$) by adding 250 μ L of oxidant solution (hypobromite iodine). The oxidation solution was gently added through the septum of the 12 ml Borosilicate glass vials using a needle to prevent the formed N_2 gas from escaping to the atmosphere.

The N_2 isotopes in the samples were then immediately quantified using the MIMS (Bay Instruments, Easton, MD, USA). The MIMS measurements involved continuous uptake of the liquid (extract) samples through a gas-permeable silicone membrane using a peristaltic pump and detecting N_2 isotopes on a quadrupole mass analyzer (Pfeiffer vacuum PrismaPlus). The N_2 isotopes were then drawn from the liquid phase by diffusion through the membrane to the mass spectrometer in a high vacuum environment. We developed a calibration curve of known $^{15}NO_3$ -N concentrations and the $(^{29}N_2/Ar)/2 + ^{30}N_2/Ar$ current ratios quantified on the MIMS (Fig. S8). The MIMS setup included a liquid N_2 trap and a reduction furnace to minimize water vapor and other dissolved gas interferences on the N_2 isotope measurements (Kana et al., 1994).

2.2.7. Quantification of potential gross nitrification and net N_2O fluxes

The isotopic pool dilution method was used to quantify the sediment and water-column potential gross nitrification rates (pGNR) (Murphy et al., 2003). For the calculation, we used the initial (T_0) and the final (T_1) $^{15}NO_3$ -N and total NO_3 -N values measured in the extracts from the initial (T_0) and final (T_1) bottles (Eq. (10)).

$$pGNR = \frac{M_i - M_f}{t} \times \frac{\log \left(\frac{H_i \times M_f}{H_f \times M_i} \right)}{\log \left(\frac{M_i}{M_f} \right)} \quad (10)$$

In the equation above, pGNR is the respective rate of gross nitrification in μ g $N g^{-1} d^{-1}$, M_i and M_f are the individual concentrations of total NO_3 -N in initial and final sediment-slurries in μ g $N g^{-1}$, H_i and H_f are the respective concentrations of $^{15}NO_3$ -N in initial and final sediment in μ g $N g^{-1}$, and t is the incubation time in days (Murphy et al., 2003).

The net N_2O fluxes during the incubations were calculated using Eq. (11), where FN_2O is the N_2O fluxes in $ng g^{-1} d^{-1}$, C_i and C_f are the respective concentrations of N_2O in initial and final sediment-slurries in

ng g⁻¹, and *t* is the incubation time in days. pGNRs and net N₂O production rates were then expressed in grams of dry sediment weight (g-SDW) after correcting for the moisture content in the sampled sediment. The moisture content was determined by drying 20 g of wet sediment in an oven at 105 °C for 24 h.

$$FN_2O = \frac{C_f - C_i}{t} \quad (11)$$

2.2.8. Quantification of functional gene abundances for nitrification and denitrification

About 1 g of sediment-slurry was sampled from the remaining slurry in each bottle after incubation to analyze the abundance of marker genes representing nitrification and denitrification processes using extracted DNA and quantitative real-time polymerase chain reaction (qPCR) (Banerjee and Siciliano, 2012). Therefore, the slurry samples were stored in sterile 1.5 ml Eppendorf tubes, frozen in liquid nitrogen, and then transferred to a deep freezer at -80 °C for later analysis (Banerjee and Siciliano, 2012). Sediment DNA samples were taken from each replicate sample of the ambient bottles and the three levels of CO₂ treatments. The DNA was extracted using the NucleoSpin Soil kit (Macherey Nagel), and yield and purity were checked with a spectrophotometer (Nanodrop, PeqLab, Germany). Following an SYBR-Green®-based (Applied Biosystems) approach with a 7300 real-time qPCR machine (Thermo Fisher Scientific, Darmstadt, Germany), qPCR runs were performed to quantify the abundance of indicator genes for ammonium oxidation (ammonia oxidation by archaea (*amoA* AOA), ammonia oxidation by bacteria (*amoB* AOB) and denitrification (*nirS*, *nirK*, and clade I *nosZ*). Previous research showed that the other *nosZ* clades were not responsive to nitrification changes, so only clade I was selected for the experiment (Duffner et al., 2021). The optimal dilution rate of the DNA extracts was fixed to 1:16 after testing for PCR inhibition. PCR reaction mixtures (25 µL) contained 12.5 µL of SYBR Green PCR Master Mix (Thermo Fisher Scientific, Darmstadt, Germany), 10 pmol of each primer, 8.5 µL of DEPC water, and 2 µL of DNA template. 2 µL of DEPC water and the mentioned reagents were used to constitute the assay negatives (no template). Serial dilutions of plasmid DNA containing the PCR products of the genes of interest (Table S2) were used to prepare the standard curves. The abundance of each marker gene was then expressed in units of copies per gram of dry sediment weight (g-SDW⁻¹). Quality was checked with electrophoreses in 1 % (w/v) agarose gels, and the dissociation-curve analysis was performed with the 7300 System SDS Software v1.3.0 (Applied Biosystems).

2.2.9. Statistical analyses

We used linear regression models on the experimental data from the eight sites to determine biogeochemical links between CO₂ oversaturation and N₂O production from nitrification-driven processes. In the models, the relationships of CO₂ concentrations against several parameters such as GNR, N₂O production rates, and the marker genes of ammonium oxidation (AOA and AOB) and nitrite (*nirS* and *nirK*) and N₂O (*nosZ*) reducers were explored. Because we hypothesized that nitrification would be dominant when DOC:NO₃ ratios are relatively low, we also examined the interaction of CO₂ and DOC:NO₃ ratios on nitrification, denitrification, and N₂O-related parameters. Similar to the meta-analysis, forest plots were used to visualize the standardized effect sizes of the fixed effects from the models ("sjPlot" and "ggplot2" packages in R version 4.3.2). To visualize the CO₂ × DOC:NO₃ interaction effects predicted from the regression models, multiple (n=10) parameter-CO₂ slope ± SE values were generated ("interactions" package in R version 4.3.2), and their bivariate relationships with changing DOC:NO₃ ratios were analyzed. Before model construction and evaluation, CO₂ and DOC:NO₃ and some independent variables were always transformed using the natural logarithm to meet the normality assumption.

3. Results

3.1. Global meta-analysis

3.1.1. Effect of in-stream CO₂ on N₂O saturation values along DOC:NO₃ ratios gradients

In-stream N₂O and CO₂ concentrations in the global dataset were mainly over-saturated (median; 407 % for CO₂ and median; 162 % for N₂O) relative to riverine concentrations in equilibrium with the atmosphere (CO₂: 421 ppm and N₂O: 0.33 ppm). The saturation levels of both gases also varied widely, spanning over 4 orders of magnitude (1–54452 % for N₂O and 3–23495 % for CO₂) (N). Similar to N₂O and CO₂ saturations, DOC:NO₃ ratios in global fluvial ecosystems were also highly variable, ranging from 0.055 – 16985. In terms of land-use influences, N₂O and CO₂ saturations were significantly (p-value < 0.05; Wilcoxon signed-rank test) higher in human-influenced cropland and urban-dominated catchments compared to forested catchments (Fig. S1). In contrast, DOC:NO₃ trends mostly followed opposite trends and were higher in forested catchments than in cropland and urban-dominated catchments (Fig. S1).

Based on the results from a linear mixed effect model, CO₂ saturation, DOC:NO₃ molar ratios, and their interaction had significant correlations with in-stream N₂O saturation (p-value<0.001, r²=0.71; Fig. S2). Increases in CO₂ saturation led to a substantial log-linear positive response in riverine N₂O saturation (effect size = 0.50, p-value<0.001). In contrast to CO₂ effects, increases in DOC:NO₃ ratios resulted in log-linear declines in N₂O saturation (effect size = -0.25 p-value<0.001; Fig. S2). The increase in DOC:NO₃ ratios also led to declines in the positive response of N₂O saturation to CO₂ saturation, with a significant negative slope at the highest DOC:NO₃ ratios (interactive effect size = -0.23; Fig. S2; Fig. 1).

The results from a multivariate structural equation model (SEM) also showed significant interactions among the direct and indirect drivers of N₂O saturation in the global data (p < 0.05; Fig. 2, Table S2). From the standardized slope parameter estimates, DOC and CO₂ positively co-varied and were negatively related to DO saturations. While DOC concentrations were positively related to NH₄-N concentrations, CO₂ saturation showed trends of a negative relationship with NH₄-N (not significant) and was additionally positively related to NO₃-N concentrations (Fig. 2). DO and NH₄-N concentrations had positive relationships with NO₃-N concentrations, whereby increase in DO and NH₄-N resulted in an up to 30 % increase in NO₃-N concentrations (Fig. 2). Direct relationships between N₂O and either CO₂ or DOC differed in magnitude and direction. An increase in CO₂ resulted in a 56 % increase in N₂O saturation. However, DOC increases had contrasting effects on N₂O, resulting in an 8 % decrease in N₂O saturation (Fig. 2). N₂O saturation was also positively related to DO and NO₃-N concentrations (Fig. 2).

3.1.2. Effect of in-vitro CO₂ enrichment on nitrification and net N₂O production

In our lab experiments, increasing CO₂ saturation was positively correlated to nitrification and N₂O-related parameters, but the relationships decreased in magnitude with increasing DOC:NO₃ ratios similar to the global dataset (Fig. 3). Within our eight experimental sites, background stream water DIN, DOC, N₂O saturation, CO₂ saturation and the DOC:NO₃ ratios ranged from 52 – 87 µmol L⁻¹, 87 – 402 µmol L⁻¹, 9 – 492 %, 196 – 1450 %, and 1.37 – 9.97, respectively. CO₂ enrichment during the sediment-slurry incubation experiments resulted in final CO₂ saturation values ranging from 498 – 4774 %, which were within the range of the global dataset and the reported range for optimum nitrifier growth rates (Denecke and Liebig, 2003).

Increases in in-vitro CO₂ saturation were positively related to increases in sediment-slurry potential gross nitrification rates (pGNRs) and the gene abundance of AOA and AOB ammonium oxidizers (effect size 0.39 – 0.47, p-value<0.001; Fig. 3 A, B). The increase in CO₂

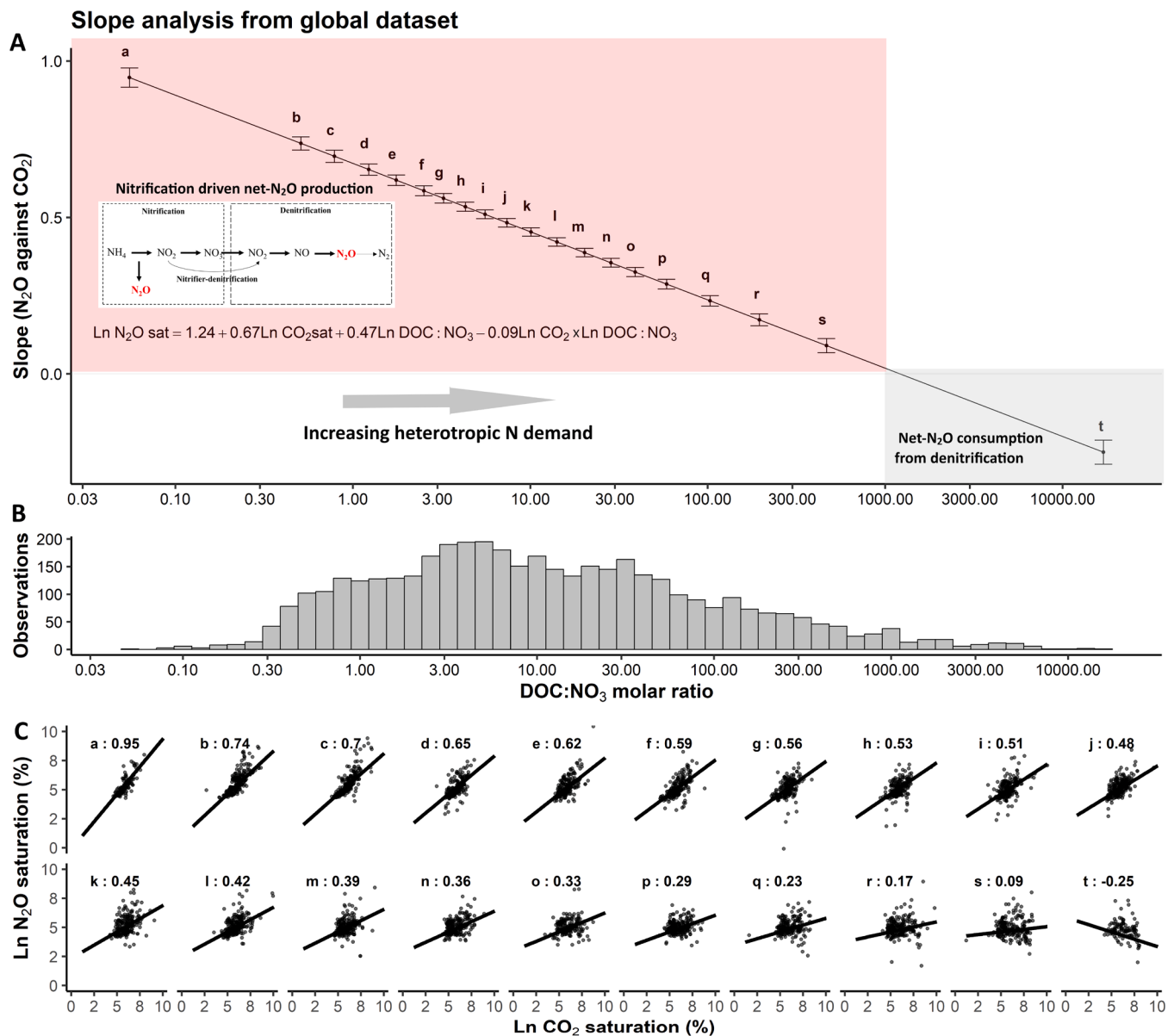


Fig. 1. **A)** Interaction plot from the linear mixed effect model (Fig. S2) illustrating the predicted non-linear decline in the slopes (p -value<0.01; $n=20$) between N_2O and CO_2 saturation levels (y-axis) with increasing $DOC:NO_3$ ratios (x-axis in log scale) in the global data. The equation on the plot outlines the log-linear relationship of the three parameters from the linear mixed-effects model (Fig. S2). The red shaded area, where the slopes are significantly positive, potentially indicates nitrification-driven N_2O production, while the grey shaded area, where slopes are significantly negative, indicates N_2O consumption from complete denitrification. The grey arrow shows increasing heterotrophic N demand with increasing $DOC:NO_3$ ratios proposed by Taylor and Townsend, 2010. Error bars on the slopes represent $\pm$ SE values. **B)** Histogram showing the distribution of $DOC:NO_3$ ratios in the global data ($N=4158$). **C)** N_2O - CO_2 scatterplots of actual field data with predicted slopes (black regression lines) across increasing $DOC:NO_3$ ratios. The predicted slopes also correspond to each slope in A, as indicated by matching lowercase letters.

saturation was also positively related to increases in N_2O saturation and net N_2O fluxes (effect size = 0.24 – 0.36, p -value<0.05; Fig. 3 C, E). However, these positive relationships with CO_2 gradually decreased in magnitude with increases in $DOC:NO_3$ ratios (interactive effect size = -0.26 – -0.39, p -value<0.05) and were insignificant at sites with $DOC:NO_3$ ratios > 5 characterized by low upstream agricultural areas (Fig. 4). Potential gross nitrification rates were positively related to *in-vitro* N_2O saturation levels (effect size 0.43, p -value<0.001; Fig S3A), but this relationship was also not significant above a $DOC:NO_3$ molar ratio of 5 (Fig. S3B). In contrast to CO_2 , N_2O and nitrification-related parameters mainly had negative relationships with O_2 and pH (Fig. S4). Denitrification potential, as indicated by the gene abundances of denitrifiers (*nirS*, *nirK*, and *clade I nosZ*), showed increases with $DOC:NO_3$ ratios

(effect size = 0.30 - 0.58, p -value<0.001, Figs. 3 D, F, 4), opposite to what was found for N_2O and nitrification related parameters. We also found that the denitrifier gene abundances mainly showed substantial positive relationships with AOB (effect size = 0.64 – 0.72, p -value<0.001), while similar relationships with AOA were not significant (Fig. S5). Overall, the relative gene abundance of ammonium oxidizers compared to denitrifiers, was significantly positively related to N_2O saturation and negatively related to $DOC:NO_3$ ratios (Fig. S6).

4. Discussion

Here, results from the meta-analysis and the experimental approach suggest a possible CO_2 fertilization effect on nitrogen cycling processes

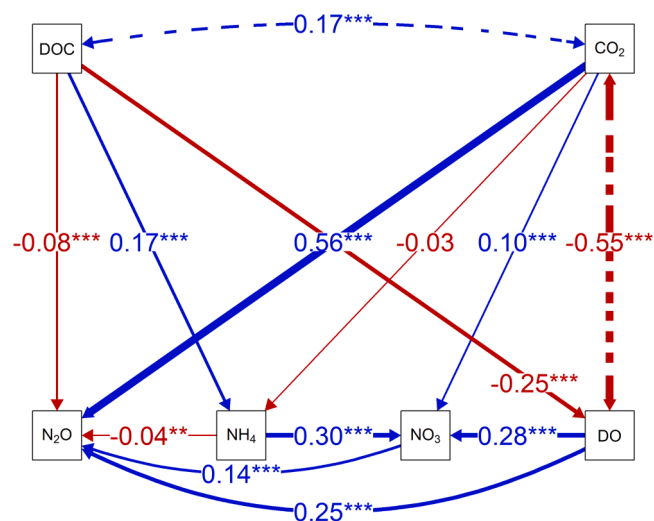


Fig. 2. Results from a structural equation model (SEM, Table S2) showing relationships between N₂O saturation and DOC ($\mu\text{mol L}^{-1}$), CO₂ (% saturation), DO (% saturation), NH₄-N ($\mu\text{mol L}^{-1}$) and NO₃-N ($\mu\text{mol L}^{-1}$) concentrations from the global dataset. Numbers show standardized slopes; blue colors represent positive relationships, and red represents negative relationships. The thickness of the line shows the strength of the relationship, and the number of asterisks represents the level of significance with * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Dashed lines indicate significant covariances in the SEM. All variables were LN transformed except for DO saturation.

responsible for net N₂O production in rivers (ammonium oxidation, nitrifier denitrification, and coupled nitrification-denitrification), which may account for the positive relationship between CO₂ and N₂O saturation values observed in most global fluvial ecosystems (Fig. 1). However, the positive effect of CO₂ fertilization on riverine N₂O production diminished at elevated DOC:NO₃ ratios, indicating that under those conditions, nitrification (a precursor process to increased N₂O production also from denitrification) may be hindered due to heightened heterotrophic NH₄ competition and oxygen limitation. (See Helton et al., 2015; Taylor and Townsend, 2010). The possible importance of nitrification in driving net N₂O production shown in this study is also consistent with a review of the results from the second Lotic Intersite Nitrogen eXperiments (LINX II; Beaulieu et al., 2011) on the controls of N₂O yields from fluvial ecosystems (Winnick, 2021). In that study, N₂O yields were negatively correlated with denitrification efficiencies and positively correlated with ammonium concentrations. This finding suggests that nitrification, which has been shown to result in higher N₂O yields due to oxygen inhibition of N₂O reduction (Meyer et al., 2008), may induce net fluvial N₂O production relative to denitrification. Wannick's (2021) findings were further corroborated in a recent study of global agricultural headwater streams, which found that ammonia-derived pathways accounted for the majority of N₂O sources in these ecosystems rather than nitrate-derived pathways (S. Wang et al., 2024).

Our study complements these studies by providing mechanistic insights into the factors driving nitrification-driven net N₂O production in global fluvial ecosystems and the potential positive climate feedback between two key greenhouse gases. As a summary of our key findings discussed in detail below, we show that nitrification-induced net-N₂O production is possibly linked to the balance between CO₂ concentrations and the DOC:NO₃ ratios, which are both influenced by anthropogenic activities (Fig. 5). In human-influenced fluvial ecosystems such as those within agricultural and urban areas, combined conditions of low DOC:NO₃ ratios and CO₂ oversaturation in them will favor net-N₂O production from enhanced nitrification rates. However, in more natural forested ecosystems with higher DOC:NO₃ ratios and lower CO₂ saturation, nitrification rates are lower, and N₂O consumption from

heterotrophic denitrification is favored (Fig. 5; Fig. S1).

4.1. The potential role of nitrification in driving the positive CO₂-N₂O relationships

Our results showed that CO₂ saturation was mainly positively related to N₂O saturation in global rivers (Fig. 1). Such a positive relationship may reflect either biogeochemical interactions between the two GHGs, as hypothesized in this study, similarities in their external sources, or a combination of both. For instance, substantial evidence that direct terrestrial inputs, particularly from urban wastewater and agricultural runoff, significantly contribute to the simultaneous loading of both N₂O and CO₂ in rivers (e.g., Mwanake et al., 2019, 2023a; W. Zhang et al., 2021). These externally sourced N₂O inputs bypass in-stream biogeochemical cycling and, therefore, do not interact with CO₂-driven N₂O production suggested in this study. To evaluate the role of biogeochemical interactions in explaining the observed positive relationship, we further investigated how this relationship varied with changing DOC:NO₃ ratios, which are known to influence nitrogen biogeochemical processes responsible for N₂O production in streams (Taylor and Townsend, 2010).

Our findings indicated that the positive effect of in-stream CO₂ on riverine N₂O saturation significantly declined with increasing DOC:NO₃ ratios, suggesting an important role of biogeochemical linkages between the two GHGs in shaping this relationship (Fig. 1). To explore how CO₂ may enhance N₂O production at low DOC:NO₃ ratios, we examined its potential role as a carbon source stimulating nitrification, a mechanism previously observed in wastewater treatment research (Jiang et al., 2015). These enhanced nitrification rates would also provide the nitrate required for N₂O production from incomplete denitrification (Cébron et al., 2005), explaining the overall net positive effect of CO₂ on N₂O in rivers. Based on the multivariate analysis, we found positive relationships of CO₂ with N₂O and NO₃ and its inverse relationship with NH₄, which suggested that the CO₂ fertilization effect on net N₂O production was possibly linked to nitrification (Fig. 2). The possible occurrence of nitrification in the global data was further supported by the positive relationships of DO and NH₄ concentrations with NO₃, as previous studies have shown that elevated oxygen and ammonium levels can promote riverine nitrification (Bernhardt and Likens, 2002; Kemp and Dodds, 2002; Webster et al., 2003). However, the positive relationship between NO₃ and N₂O may still imply that N₂O production is solely dominated by denitrification (e.g., Baulch et al., 2011; Beaulieu et al., 2011; Herreid et al., 2021), and we, therefore, required additional evidence linking net N₂O production to N cycling processes driven by increased nitrification rates. This evidence was drawn from our study's meta-analysis and incubation experiments. From the meta-analysis, we observed a negative effect of DOC, the primary carbon source for heterotrophic denitrification, on N₂O saturation in rivers, while CO₂, the primary carbon source for nitrifiers, showed strong positive relationships with N₂O saturation (Fig. 2). This finding suggested that while CO₂-driven nitrification may be responsible for net N₂O production, conditions favoring denitrification may result to both N₂O production and consumption, as increased heterotrophic N demand under elevated DOC concentrations may lead to N₂O reduction to N₂ through complete denitrification (Quick et al., 2019).

Our incubation experiments provided further empirical evidence for the role of nitrification in driving net N₂O production in rivers, based on the availability of its primary carbon source, CO₂. At streams with DOC:NO₃ ratios <5, mainly located within agricultural areas, the experimental results supported the circumstantial evidence from the global data, showing that sediment and water column potential gross nitrification rates, the abundance of ammonium oxidizers, N₂O saturation, and net N₂O production rates increased with elevated CO₂ (Figs. 3 and 4). N₂O saturation levels at these sites were also positively related to the potential gross nitrification rates (Fig. S3). Overall, the experimental and meta-analysis findings both suggested that increased nitrification

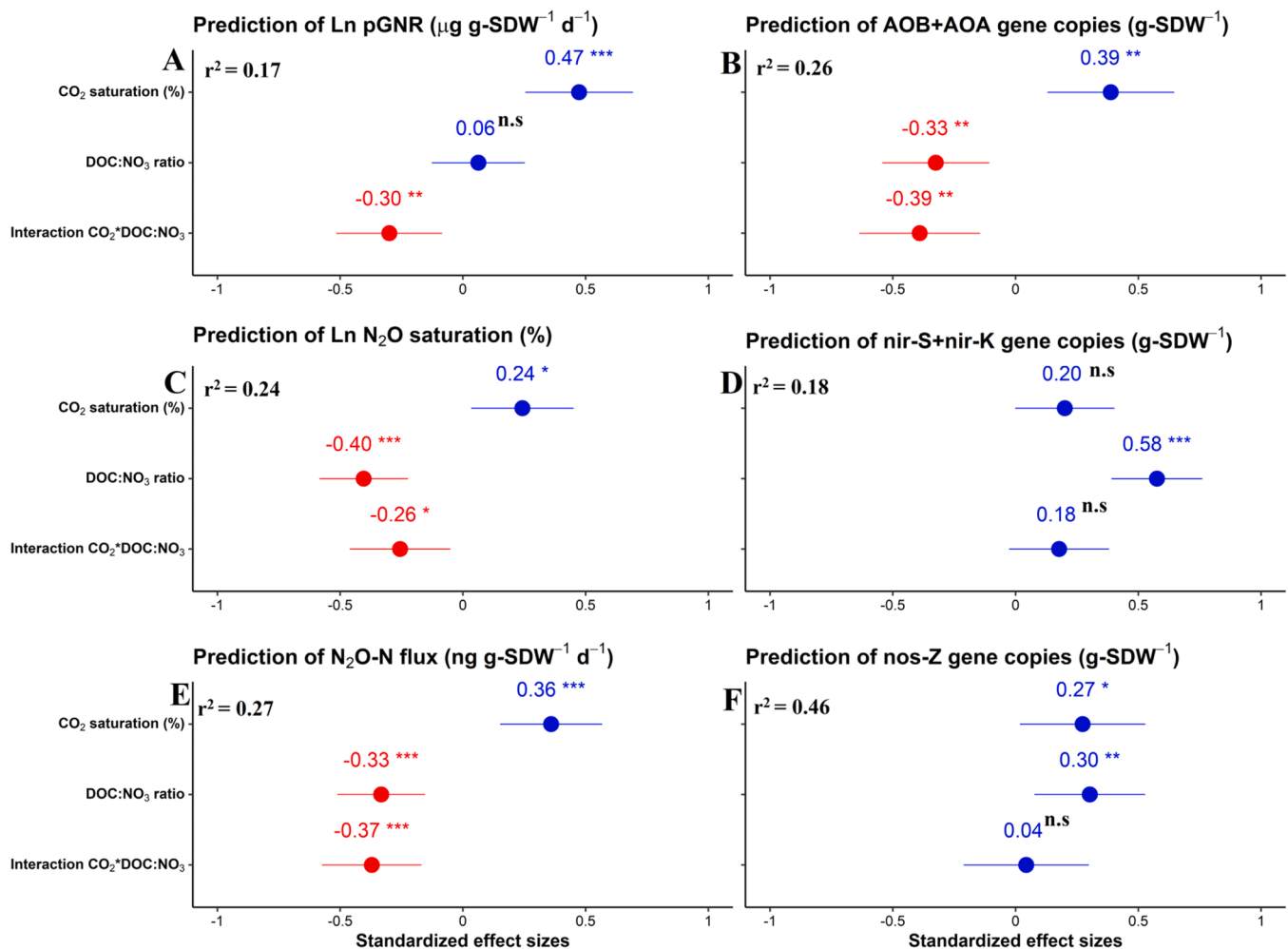


Fig. 3. Forest plots showing effect sizes from the experimental data where CO₂ saturation was manipulated in sediment slurries from 4 forested, 2 pasture, and 2 cropland-dominated streams, which varied with respect to background DOC:NO₃ ratio (see experimental setup, methods). Effect sizes of CO₂, DOC:NO₃ ratios and their interactions are shown for **A**) potential gross nitrification rates; **B**) gene abundance for ammonium oxidizers (amoA AOA; amoB AOB); **C**) N₂O % saturations; **D**) gene abundances of nitrite reducers (*nirS* and *nirK*); **E**) net N₂O production rates; and **F**) gene abundance of nitrous oxide reducers (clade I *nosZ*). Numbers show the standardized slope values from linear regression models, with blue showing positive and red showing negative relationships. Horizontal lines on the dots represent the confidence interval of the effect sizes (CI=95 %). Significance is shown by the number of asterisks * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

rates induce net N₂O production under low DOC:NO₃ ratios and high CO₂ saturation, which mainly characterizes human-influenced rivers (Fig. 5). However, the positive effects of CO₂ oversaturation on net N₂O production declined with increasing DOC:NO₃ ratios, similar to what we found in the global data (Fig. 1). We attribute this finding to reducing redox conditions and increased heterotrophic N demand under elevated DOC:NO₃ ratios (e.g., Helton et al., 2015; Taylor and Townsend, 2010), which support N₂O consumption by complete denitrification rather than its production from nitrification or incomplete denitrification. This conclusion also aligns well with the antagonistic relationship we observed between DOC:NO₃ ratios and the relative gene abundance of ammonium oxidizers compared to denitrifiers (*nirS*, *nirK*, and clade I *nosZ*), which was positively correlated with N₂O saturation (Fig. S6). Our findings, therefore, align with previous fluvial studies reporting overall higher N₂O yields from nitrification than from denitrification, likely due to the latter's role in also facilitating N₂O reduction under certain environmental conditions (S. Wang et al., 2024; Winnick, 2021). The dual role of denitrification was also shown in a recent global meta-analysis investigating the drivers of N₂O emission factors from rivers, where total N₂O yields from denitrification were significantly negatively correlated with DOC:NO₃ ratios (J. Wang et al., 2022). The authors attributed this negative correlation to the fact that labile carbon promotes both N₂O production and reduction via denitrification;

however, the direction of this relationship depends on the nitrogen status of the riverine ecosystem (Quick et al., 2019). In nitrogen-limited riverine ecosystems, particularly in forested catchments, complete denitrification may result in rivers acting as net N₂O sinks (e.g., Aho et al., 2023; Borges et al., 2019; Mwanake et al., 2025). For instance, this mechanism has been inferred in tropical forest streams in South America and Africa, where high DOC concentrations and reducing redox conditions likely promote N₂O undersaturation through its conversion to N₂ via complete denitrification (Borges et al., 2019; Chiriboga and Borges, 2023). In contrast, nitrogen-rich riverine ecosystems, such as those in urban areas, often exhibit net N₂O production as a result of incomplete denitrification (e.g., Chen et al., 2025; C. Wang et al., 2024).

Although CO₂ oversaturation showed no significant positive effect on potential gross nitrification and N₂O production at sites with >5 DOC:NO₃ ratios, we did find a positive effect of CO₂ on the abundance of denitrification genes (Fig. 3). We contend that the stimulation of ammonium oxidizers by CO₂, particularly AOB that showed the most substantial positive relationship with the abundance of denitrifier genes (*nirS*, *nirK*, and clade I *nosZ*) (Fig. S5), may explain our findings. AOB may be more sensitive to increasing in-stream CO₂ concentrations than AOA as it cannot directly fix the more abundant inorganic carbon form (HCO₃⁻) and is known to conduct denitrification under reducing redox conditions (Cooper et al., 1969). These results are consistent with

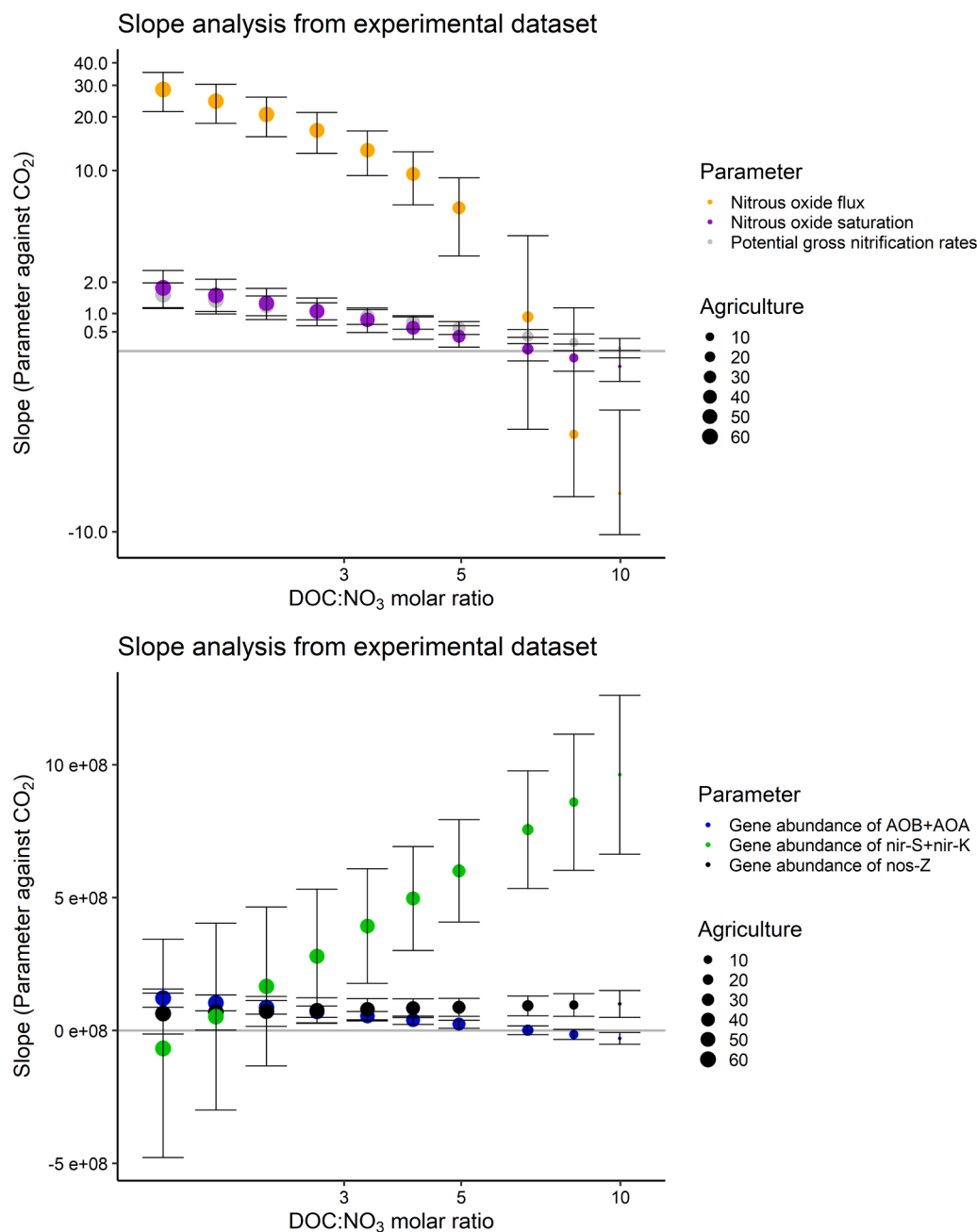


Fig. 4. Interaction plot showing model results from the experimental data set (Fig. 3) relating the slopes between several parameters and CO₂ saturation (y-axis) against DOC:NO₃ ratios (x-axis in log scale). The y-axis shows the slopes of process rates (Panel A) and gene abundances (Panel B). Error bars represent $\pm$ SE values ($n=10$) along increasing DOC:NO₃ ratios. The size of the dots indicates the percent of the sub-catchment that comprises agricultural land use.

previous laboratory tracer studies, which demonstrated the existence of nitrifier denitrification by AOB (Jung et al., 2014; Stieglmeier et al., 2014; Wrage-Mönnig et al., 2018). Similar findings were also recorded in agricultural headwater streams in China, whereby metagenome-assembled genomes (MAGs) analyses indicated that nitrifying bacteria, including AOB, contain greater abundances of N₂O production-related genes than denitrifying bacteria (S. Wang et al., 2024). Based on these findings, we contend that enhanced nitrification rates from CO₂ fertilization may increase the dominance of nitrification over denitrification on net N₂O fluxes due to additional N₂O yields from AOB-mediated nitrifier denitrification.

4.2. Disentangling CO₂ effects from covariates such as O₂ and pH

Even though previous studies have also found that increased respiration can lead to increased N₂O fluxes from rivers (e.g., Beaulieu et al., 2011; Madinger and Hall, 2019; Reisinger et al., 2016), most of them have linked it to oxygen depletion during the process that favors N₂O production via denitrification (e.g., Rosamond et al., 2012). Our study's results offer an alternative explanatory mechanism, as CO₂ saturation, a product of respiration, showed more meaningful biogeochemical stimulations of N₂O production than O₂ and even pH (Fig. S4). In all eight experimental sites, we found either negative relationships between gross nitrification rates and the gene abundances of ammonium oxidizers with O₂ and pH or no relationships (Fig. S4). These relationships contradict the expected positive relationships with both variables, as nitrification is

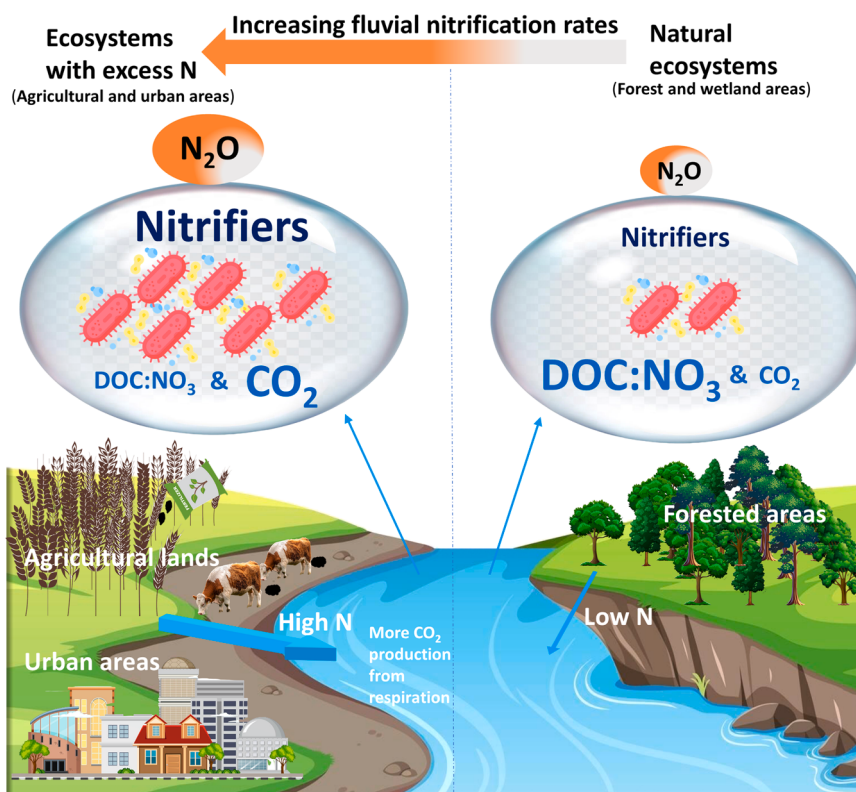


Fig. 5. Conceptual diagram outlining the mechanism of how relatively high in-stream CO_2 concentrations and low $\text{DOC}:\text{NO}_3$ ratios due to human activities result in enhanced nitrification-driven N_2O production in fluvial ecosystems (see Fig. S1). The size of the text indicates the magnitude of change in each parameter due to anthropogenic activities. The color gradient for in-stream N_2O concentration estimates the contribution of nitrification (red) relative to denitrification (grey) inferred from gene abundance trends in this study (Fig. S6) and the results of Wang et al. (2024).

an aerobic process known to increase with pH (e.g., Bernhardt and Likens, 2002; Huesemann et al., 2002). Based on these findings, CO_2 fertilization of ammonium oxidation and the complete nitrification process offered an alternative argument in explaining our results. Even at streams with relatively high $\text{DOC}:\text{NO}_3$ ratios, where heterotrophic denitrifiers may dominate due to stoichiometric advantages and reduced redox conditions, CO_2 saturation showed more substantial positive effects on the abundances of functional denitrification genes than O_2 (Fig. 4, S4). This result further suggests that improved nitrite/nitrate production with elevated CO_2 rather than declines in O_2 concentrations may also drive N_2O production from the nitrifier denitrification processes carried out by AOB or denitrifiers.

4.3. Possible implications of the CO_2 effect on global riverine N_2O emissions

Recent research indicates that unaccounted global night-time riverine CO_2 emissions are, on average, 27 % higher than daytime emissions, with the most significant differences (median: ~40 %) occurring in open-canopy streams with low DOC concentrations (Gómez-Gener et al., 2021), typical of cropland and urban streams that are known N_2O hotspots (e.g., Mwanake et al., 2025; Mwanake et al., 2023a; Xu et al., 2024). Using the log-linear biogeochemical relationship derived from the global dataset (Fig. 1A) and Monte Carlo simulations for uncertainty assessment ($n=1000$), we evaluated the potential impact of elevated nighttime CO_2 on riverine N_2O emissions in these streams at varying levels of $\text{DOC}:\text{NO}_3$ ratios. Given the positive relationship between greenhouse gas saturation and emissions, accounting for underestimated nighttime CO_2 emissions suggests that global N_2O emissions from streams could be 12 % (Interquartile range: 8 – 15) higher than current estimates. Although similar underestimations of riverine N_2O fluxes have been reported in site-specific studies (e.g., Piatka et al.,

2024; Woodrow et al., 2024), this study uniquely highlights their potential significance on a global scale.

Additionally, our study suggests that the CO_2 fertilization effect on global riverine N_2O production could become a significant climate feedback mechanism with land use changes. This is because projections of global land use changes under different climate scenarios indicate substantial expansion of fertilizer-intensive croplands and urban areas by the end of the 21st century (e.g., Alexander et al., 2018; Li et al., 2019). Conceptually, this expansion is expected to lower in-stream $\text{DOC}:\text{NO}_3$ ratios and increase CO_2 concentrations, creating favorable conditions for high N_2O production rates from nitrification-driven processes (Fig. 5). Several studies have already shown that increased nitrogen inputs into rivers from cropland or urban-dominated catchments lower riverine C: N ratios (Wachholz et al., 2023), increase in-stream CO_2 concentrations (Xu et al., 2024), and result in higher contributions of nitrification to net N_2O production than denitrification (S. Wang et al., 2024). To calculate the potential impacts of land-use transitions on riverine N_2O emissions, we also applied the log-linear biogeochemical relationship generated in this study (Fig. 1A) and used Monte Carlo simulations ($n=1000$) for uncertainty assessment. For instance, using the land use-specific global range values of CO_2 saturation and $\text{DOC}:\text{NO}_3$ ratios (Fig. S1), a transition from forested catchments to cropland-dominated catchments results in a 45 % (IQR= -4 – 124) increase in riverine N_2O emissions. A more pronounced increase of 111 % (IQR= 18 – 297) in N_2O emissions is anticipated with shifts toward urban-dominated catchments characterized by higher CO_2 saturations and lower $\text{DOC}:\text{NO}_3$ ratios (Fig. S1). These land use change effects are amplified when accounting for unmeasured nighttime CO_2 increases in urban and cropland-dominated streams described above. Under these corrected CO_2 conditions, riverine N_2O emission is predicted to rise by 52 % (IQR= -12 – 170) for transitions from forest to cropland

catchments and by 161 % (IQR = 21 – 473) for shifts from forest to urban catchments. Based on these corrected values, we argue that as land use intensifies, mainly through agricultural expansion and urbanization from natural lands, these shifts in biogeochemical factors that regulate nitrification could substantially increase global riverine N₂O emissions.

4.4. Limitations of the study and targets for future research

While we were able to show that the positive CO₂ to N₂O relationship observed in global fluvial ecosystems may be related to enhanced nitrification in human-influenced rivers with low DOC:NO₃ ratios, our study was not without limitations. For example, the global dataset used to test some of the key hypotheses in this study includes sites sampled at different times and locations. Although the variance introduced by the nature of the data was accounted for in the mixed-effects models, questions remain as to whether the CO₂ fertilization effect on N₂O production is higher in certain regions or varies seasonally. The lack of process-based measurements on a global scale also means that the evidence from our global meta-analysis is primarily circumstantial, with factors such as similar sources for CO₂ and N₂O potentially playing an important role in explaining their positive relationship. Furthermore, it is still unclear based on our *in vitro* experiments how much of the N₂O produced originates directly from hydroxylamine oxidation or other nitrification-induced processes, such as incomplete denitrification and nitrifier denitrification (Jung et al., 2014; Stieglmeier et al., 2014; Wrage-Mönnig et al., 2018). The magnitude of the possible climate feedback also remains uncertain despite its potential global relevance. This is due to difficulties in predicting future riverine CO₂ and DOC:NO₃ trends, which may be impacted by drivers other than land use changes, such as climate change. Therefore, we suggest that future global studies need to be informed by advanced experiments such as using stable isotope approaches and microbial gene analysis to answer some of the questions above, primarily as the drivers and processes regulating N₂O production and consumption in rivers still remain uncertain relative to those of CO₂ and CH₄.

5. Conclusions

- The positive CO₂ effect on nitrification-driven net N₂O production from fluvial ecosystems demonstrated in this study may signify an important climate feedback mechanism.
- Future land use changes from natural ecosystems to cropland or urban areas may further enhance the effects of this climate feedback mechanism, thereby increasing the contribution of fluvial ecosystems to global GHG budgets.
- More research is needed to better understand how elevated instream CO₂ affects N₂O production processes in streams and the implications of these changes under global climate change.

Data and code availability

The compiled global and experimental data used in this study will be available upon request. This study's statistical analysis and figures were performed and produced with publicly available packages in R (version 4.3.2).

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CRediT authorship contribution statement

R.M. Mwanake: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **G.M. Gettel:** Writing – review & editing, Supervision, Conceptualization. **E.G. Wangari:** Writing – review & editing, Data curation. **G.W. Macharia:** Writing – review & editing, Data curation. **R. Martínez-Cuesta:** Writing – review & editing, Data curation. **S. Schulz:** Writing – review & editing, Resources, Methodology. **M. Schlöter:** Writing – review & editing, Resources, Methodology. **K. Butterbach-Bahl:** Writing – review & editing, Supervision, Conceptualization. **R. Kiese:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.watres.2025.124320](https://doi.org/10.1016/j.watres.2025.124320).

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