

## #Reviewer 2

In general, this is a very nice study design and thorough sampling and analysis. I do not see any issues in that regard. While most comments are minor, it does seem that the introduction and discussion sections have received less attention, and are currently quite superficial in some sections, and overlook or mix up some concepts/terminology. So while I like this study overall, my comments below highlight the need for the authors to dig a bit deeper in their framing and interpretation of the work relative to other work in the field. I support this paper being published if the authors can fix these issues.

General comments:

Issue with lateral inputs in introduction: Up to line 62, nowhere in the paper to this point is lateral transfer of GHGs mentioned. It is especially important for CO<sub>2</sub> in headwater systems, but in the drainage ditches, the GHG production in the wetlands themselves must be a huge fraction of the emissions budget, no? The introduction needs to more thoroughly reflect this aspect of mechanistic control.

**Response:** Thank you for the critical observation and suggestion for improvement. We have edited the introduction's first paragraph to better explain the mechanisms driving GHG seasonal and spatial dynamics in headwaters. We have also acknowledged the contribution of terrestrial soils to GHG dynamics within drainage ditches in our introduction (See response to a later comment and suggestion)

"Several biogeochemical processes are responsible for GHG production and consumption within headwater ecosystems. CO<sub>2</sub> production is mainly attributed to the respiration of organic matter (Battin et al., 2008). Production of CH<sub>4</sub> occurs through methanogenesis, with carbon dioxide and acetic acid as substrates under anaerobic conditions (Stanley et al., 2016). Methane consumption is also possible through methanotrophy in oxygen-rich stream waters, producing CO<sub>2</sub> (Shelley et al., 2014). N<sub>2</sub>O is mainly a byproduct in nitrification (under aerobic conditions) or an intermediate product in denitrification (under anaerobic conditions), but it can also be reduced to N<sub>2</sub> in organic-rich and nitrate-poor ecosystems (Quick et al., 2019). Apart from instream biogeochemical production, GHG concentrations in headwater streams may also originate from external sources such as groundwater and terrestrial soils (e.g., Borges et al., 2015; Hotchkiss et al., 2015). These external sources are generally dominant during periods of heavy precipitation when the hydrological connectivity between the streams and their surrounding terrestrial landscape and groundwater is activated. Yet, partitioning the sources of these GHGs between in-situ production and external sources remains a challenge, as their contributions are mainly compounded and also vary widely depending on discharge conditions and the surrounding land use (e.g., Aho & Raymond, 2019; Borges et al., 2019; Mwanake et al., 2022)."

The overview paragraph in the intro line 75 and on is pretty thin on mechanistic insight. Please integrate lateral inputs of GHG more carefully, aside from one sentence, it is all about internal production. How do storms and seasonal changes in hydrology alter the balance between catchment CO<sub>2</sub> loading and internal production?

**Response:** Thank you for the critical observation and suggestion for improvement. We have added sentences in the introduction to indicate times where seasonal variabilities in discharge control in situ vs external GHG sources.

"Seasonality in precipitation regulates discharge, whereby heavy precipitation events or snowmelt during spring can result in high discharge events. At the same time, dry summers and winter periods are often characterized by lower discharge (e.g., Aho et al., 2022). Previous studies have shown that low discharge periods with longer water residence times favor instream GHG production processes (e.g., Borges et al., 2018; Mwanake et al., 2022). In contrast, high discharge periods with shorter water residence times are unfavorable to *instream* C and N cycling, resulting in the dominance of externally sourced GHGs from upstream terrestrial sources depending on the surrounding land use. For example, studies have found that during high discharge periods, streams draining wetlands show peak CO<sub>2</sub> and CH<sub>4</sub> concentrations (e.g., Aho et al., 2019; Borges et al., 2019), and pronounced N<sub>2</sub>O concentrations are found in streams of cropland-dominated catchments (e.g., Mwanake et al., 2022)."

Discussion section 4.3: This section should be expanded. We need a more thorough numerical comparison with other studies. There needs to be a conclusion, what is special or new about your study compared to those papers? Anything unique here? What can you say overall about the land use sites vs non? I think that more work needs to be done in this section to take what is a very nice dataset and analysis, and actually make it impactful in terms of the insights that you are providing the community.

**Response:** Thank you for the critical observation and the suggestions for improving our discussion. We have expanded our discussion section to include a first paragraph that reflects on our key findings from this study, complementing what is currently in the conclusion section. We have also added a comparison of our flux estimates with current global synthesis datasets but limited the site-specific comparisons to the temperate region, which better represents the climatic as well as land management practices of our study area.

## Discussion

"The GHG fluxes quantified from headwater streams and ditches in this study add to the growing evidence that both aquatic ecosystems are significant net emitters of GHGs to the atmosphere. In agreement with previous studies, CO<sub>2</sub> accounted for most (>81 %) of the annual fluvial GHG fluxes in CO<sub>2</sub> equivalents (e.g., Marescaux et al., 2018; Mwanake et al., 2022; Li et al., 2021). However, the presence of upstream agricultural and settlement areas seemed to alter these trends by reducing the contribution of CO<sub>2</sub> and increasing N<sub>2</sub>O and CH<sub>4</sub> contributions. The effects of the above anthropogenic activities on aquatic GHG dynamics were twofold. Drainage ditches were landscape hotspots for CH<sub>4</sub> emissions while increasing upstream agricultural and settlement areas resulted in fluvial N<sub>2</sub>O hotspots. The emissions from human-influenced streams were further supplemented by wastewater inflows, which provided year-long nutrients, labile carbon, and GHGs supplies, resulting in much higher CO<sub>2</sub> and N<sub>2</sub>O annual emissions. Besides influencing GHG hotspots, the temporal dynamics of GHG fluxes from streams and ditches in our study were further impacted by anthropogenic influences. While catchments dominated by wetlands or forested areas exhibited low seasonal variabilities due to limitations in conditions that favor peak emissions (increased gas transfer velocities and sufficient GHG supplies), opposite trends were found at catchments dominated by agricultural and settlement areas or affected by wastewater inflow. These findings suggested that the occasional peak GHG emissions in the later catchments

represented periods where external GHG sources from supersaturated terrestrial soils or wastewater inflows outweighed supply constraints during peak discharge periods with high gas transfer velocities. These findings suggest that future land use changes from natural forests to agricultural and settlement areas may increase the radiative forcing of aquatic GHG emissions by increasing the magnitudes of their annual fluxes, especially in a changing climate with more extreme discharge conditions."

"This study's daily CH<sub>4</sub> and N<sub>2</sub>O diffusive flux ranges from both streams and ditches are mostly within the same order of magnitude as those previously reported in global synthesis studies (Table 3: Hu et al., 2016; Stanley et al., 2016). In contrast, this study reported among the highest fluvial CO<sub>2</sub> emissions compared to other regional and global studies, with significant mean fluxes of up to 51 g-C m<sup>-2</sup> d<sup>-1</sup> (Table 3). We attribute this finding to moderate-steep slopes such as those quantified in the mountain streams of the Loisach catchment or diffuse and point terrestrial dissolved CO<sub>2</sub> inputs from the more human-influenced Schwingbach and Neckar catchments, translating to higher fluvial CO<sub>2</sub> fluxes (Fig. 6)."

## Conclusion

"Streams and ditches in agricultural and settlement areas were characterized by significantly higher GHG fluxes with more significant intra-annual variabilities than forests and wetlands. A combination of wastewater inflows and agricultural land use resulted in the highest fluvial CO<sub>2</sub> and N<sub>2</sub>O fluxes, particularly during high discharge periods with substantial external dissolved GHGs. In general, anthropogenic activities resulted in a potential breakdown of the expected decrease of the GHG source strengths with increasing stream order, as higher-order streams in the Neckar sub-catchments with cropland and settlement influences had either higher or comparable concentrations and fluxes than small streams in the Loisach and Schwingbach catchments. As most studies use stream order to upscale local and regional riverine fluxes, we show from our results that caution must be taken in applying the methodology, particularly across catchments differing in land use intensity.

Our findings indicate that future work should focus more on human-influenced headwater stream ecosystems, as they contribute disproportionately large annual fluxes and are more temporally variable than natural ones. Our study also found higher winter N<sub>2</sub>O fluxes, emphasizing the need for continuous sampling regimes covering full years to reduce uncertainty in annual GHG emission estimates. Combining continuous sampling regimes of all three biogenic GHGs (CO<sub>2</sub>, N<sub>2</sub>O, and CH<sub>4</sub>) across catchments with contrasting land uses will further constrict riverine emissions and aid in developing targeted emission reduction mitigation strategies."

Line by Line comments:

L 29-31 - is it in situ, or is the ditch draining a landscape that has a lot of GHG production? This would be adjacent source, not in situ in the ditch.

**Response:** Thank you for your questions. Both sources can be significant as the ditches also have deep sediment layers dominated by particulate OM, which may favor internal production too. We have edited the line to reflect the existence of both sources.

"Besides draining CH<sub>4</sub> and CO<sub>2</sub>-rich terrestrial soils, drainage ditches are characterized by short water residence times, high organic loads, and highly variable O<sub>2</sub> levels, which can simultaneously support vigorous CH<sub>4</sub> and CO<sub>2</sub> production and, subsequently, higher fluxes."

L33-34 - but natural systems make up a huge fraction of the total global stream area, so if your goal is to simply scale the contribution of streams, then your reasoning is not accurate. I'd refocus the implications here and say something more generalizable about why emissions research in these impacted rivers are important.

**Response:** Thank you for your critical comment and suggestion. We have rephrased this line to reflect on the importance of studying human-influenced streams.

"Therefore, future studies should focus on anthropogenically perturbed streams, as their GHG emissions are much more variable in space and time and can potentially introduce the largest uncertainties to fluvial GHG estimates."

L37 – In this figure, No indirect effects on GHG in this diagram in anthropogenic domain. Nutrients, hydrology, etc will also modify emissions patterns indirectly.

**Response:** Thank you for the observation. The indirect effects are included in the above-ground runoff and point wastewater sources indicated by the blue arrows.

L43 - photochemical DOM processing too.

**Response:** Thank you for the suggestion. It's true that photochemical processing also leads to CO<sub>2</sub> production. However, we were only interested in the primary biogenic process, respiration, as we aimed to relate it to the substrate and oxic levels within the lotic ecosystems. We have edited the sentence to reflect this.

"Biogenic CO<sub>2</sub> production is mainly attributed to respiration of organic matter (Battin et al., 2008)."

L48 – conclusion sentence needed.

**Response:** Thank you for the suggestion. We have edited the paragraph to include a conclusion.

"Yet, partitioning the sources of these GHGs between in-situ production and external sources remains a challenge, as their contributions are mainly compounded and also vary widely depending on discharge conditions and the surrounding land use (e.g., Aho & Raymond, 2019; Borges et al., 2019; Mwanake et al., 2022)."

L58 – compared

**Response:** Redone.

L69 – numerical context needed.

**Response:** Thank you for the observation. We have added the numerical context of the contributions.

"For example, in a study of urban-impacted rivers in the Seine basin in France, Marescaux et al. (2018) found elevated CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O concentrations and fluxes downstream of wastewater inflows, which disproportionately contributed up to 52 % of the basin-wide annual GHG fluxes."

L70 – in rivers linked to land use, or direct land use emissions? Be specific here.

**Response:** Thank you for your critical comment. We have rephrased the sentence to reflect stream emissions and not from the terrestrial landscape.

"Similar findings were also found in urban-impacted rivers in China, where GHG emissions were up to 14 times higher than those in other land uses (Zhang et al., 2021)."

L64-74 - here, integrating the important discussion points from key articles would strengthen your introduction. I list a few but there are also more:

Park JH, Nayna OK, Begum MS, Chea E, Hartmann J, Keil RG, Kumar S, Lu X, Ran L, Richey JE, Sarma VV. Reviews and syntheses: Anthropogenic perturbations to carbon fluxes in Asian river systems—concepts, emerging trends, and research challenges. *Biogeosciences*. 2018 May 17;15(9):3049-69.

Begum MS, Bogard MJ, Butman DE, Chea E, Kumar S, Lu X, Nayna OK, Ran L, Richey JE, Tareq SM, Xuan DT. Localized pollution impacts on greenhouse gas dynamics in three anthropogenically modified Asian river systems. *Journal of Geophysical Research: Biogeosciences*. 2021 May;126(5):e2020JG006124.

**Response:** Thank you for sharing the references. We have adjusted the paragraph to add more context and updated the suggested references.

"In fluvial ecosystems within settlement areas, point-source inflows of wastewater effluents have also been reported to alter natural GHG trends along the river continuum (Park et al., 2018). The wastewater effluent is either substrate-rich, favoring insitu GHG production, or GHG-rich, resulting in high riverine GHG emissions downstream of the inflow point (e.g., Marescaux et al., 2018; Begum et al., 2021; Zhang et al., 2021; Wang et al., 2022). For example, in a study of urban-impacted rivers in the Seine basin in France, Marescaux et al. (2018) found elevated CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O concentrations and fluxes downstream of wastewater inflows, which disproportionately contributed up to 52 % of the basin-wide annual GHG fluxes. Similar findings were also found in urban-impacted rivers in China, where their GHG emissions were up to 14 times higher than those in other land uses (Zhang et al., 2021). Yet, studies on GHG emissions from urban-impacted fluvial ecosystems are still scarce, and therefore their contributions to riverine annual GHG budgets are not well constrained. Moreover, little is known about the cumulative effects of diffuse and point pollution sources on the magnitude of riverine GHG fluxes and whether the diffuse pollution sources exert longer-lasting controls on their fluxes than the point sources."

L72 – constrained

**Response:** Redone.

L74 – through this part of the intro, this terminology or contrasting of urbanization and land use is confusing. Do you mean point- and non-point pollution impacts? Or by land use do you mean agricultural land use specifically? Please clarify.

**Response:** Thank you for the critical comment. We meant point and non-point sources in this context. We have rephrased the sentence to generally reflect this view.

"Moreover, little is known about the cumulative effects of diffuse and point pollution sources on the magnitude of riverine GHG fluxes and whether the diffuse pollution sources exert longer-lasting controls on their fluxes than the point sources."

L89 - this point could be reworded. It is essentially saying that low sampling frequency does not capture short term variability in GHG cycling. This is obvious, so could you take this line of thinking a step further?

**Response:** Thank you for the critical comment. We reworded the sentence and took it a bit deeper into the gaps to be addressed in our study.

"The dynamic interactions between seasonality and land use discussed above indicate that less frequent measurements of riverine GHG concentrations and fluxes may fail to capture periods of elevated fluvial emissions at spatially hotspot areas, resulting in an underestimation of the annual emissions."

L91 (and more general) - this statement is not exactly defensible. People have been measuring stream GHG patterns for decades. Note that the oldest reference cited here is 2018. A deeper exploration of the literature here would be needed to pinpoint more specific unknowns about riverine emission patterns. At the same time, much of the issues in this pgph is already presented in the last one discussing sub-annual patterns. Consider just removing or really going much deeper.

**Response:** Thank you for the critical comment. We reworded the paragraph to outline better our contribution to the already existing knowledge pull.

"The dynamic interactions between seasonality and land use discussed above indicate that less frequent measurements of riverine GHG concentrations and fluxes may fail to capture periods of elevated fluvial emissions at spatially hotspot areas, resulting in an underestimation of the annual emissions. Yet, only a handful of studies in temperate streams have assessed the seasonal dynamics of GHG fluxes at sampling points with contrasting land uses (e.g., Marescaux et al., 2018; Borges et al., 2018; Herreid et al., 2021; Galantini et al., 2021), resulting in limitations when analyzing the mechanisms that drive either hot periods or hotspots of fluvial GHG fluxes. As climate change causes more extreme discharge conditions and as agricultural intensification and settlement areas continue to increase (Winkler et al., 2021), more studies that cover a wide array of land uses, discharge, and temperature conditions are needed to allow developing better mechanistic understanding of their effects on fluvial GHG dynamics by unraveling synergistic or antagonistic relationships amongst them. These increased process understanding will form the basis of future mechanistic modeling approaches, which are essential to predict better how fluvial GHG emissions will respond to future climate and



land use changes (Battin et al., 2023)."

L209 – detail standards used for GC calibration.

**Response:** Thank you for the critical comment. We have added in the methods the calibration standards used for the GC.

"The standards used for the GC calibration were 450, 800, 1000, 1500, 2000, and 3000 ppm for CO<sub>2</sub>, 1, 2, 3, 4, 5, and 6 ppm for CH<sub>4</sub> and 0.4, 0.8, 1, 1.5, 2, and 3 ppm for N<sub>2</sub>O."

L278 - Terminology "end" and "exogenous is misleading throughout, because things like water temperature are within the system, so to me are not exogenous. Why not call them 'substrate' and 'environmental conditions' or something? This comment applies throughout the paper where this framework is used.

**Response:** Thank you for your suggestion. The terminologies are generic with the SEM framework. However, as suggested, we have adopted substrate and environmental conditions to replace the endo and exogenous, respectively.

"Path analysis from structural equation models (SEMs, "lavaan" package in R version 4.1.1) was used to determine how environmental factors linked to seasonality and land use directly or indirectly influenced *instream* GHG production and consumption processes as well as external GHG sources, i.e., dissolved GHG inputs to the streams originating from either wastewater inflows or terrestrial landscapes which were not produced *in situ*. In brief, these SEMs were constructed based on causal relationships between environmental variables (interpreted as ultimate drivers of GHG concentrations) and substrate variables, which are affected by the environmental variables and also act as immediate drivers that affect GHG concentrations. Substrate variables in the models, which are known to influence *in situ* biogeochemical GHG production and consumption processes directly, included dissolved oxygen DO (% saturation), DOC (mg L<sup>-1</sup>), NH<sub>4</sub>-N (mg L<sup>-1</sup>), and NO<sub>3</sub>-N (mg L<sup>-1</sup>) concentrations (Battin et al., 2008; Stanley et al., 2016; Quick et al., 2019). The environmental variables in the models, which influence *in situ* GHG concentrations either directly by facilitating dissolved GHG inputs or indirectly by controlling the substrate variables, were water temperature (°C) (a proxy for different seasons), stream velocity V (m s<sup>-1</sup>), % upstream agricultural area for each sampling point (AGR: grassland + cropland area) and wastewater inflows (WW: Boolean numbers, i.e., 1 for the presence of wastewater inflow and 0 for absence)."

L305 – fold

**Response:** Redone

L337 - throughout the water chem and GHG results sections, when something is significant or not, please report the test statistic in the text.

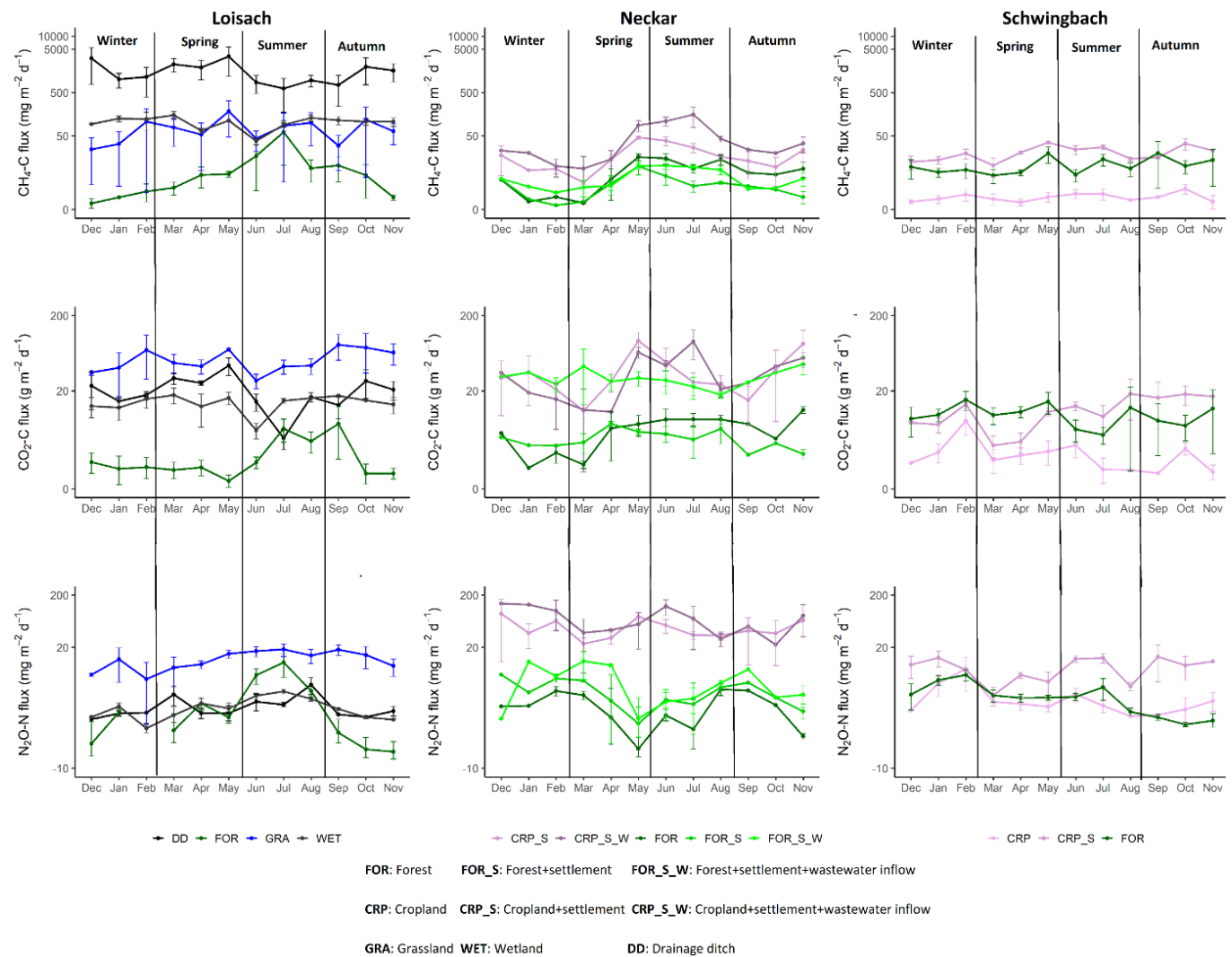
**Response:** Thank you for your critical observation and suggestion. We have included the test statistic in all our results in the main text. See examples below

"Seasonality had an overall significant effect ( $p < 0.05$ ) on stream velocities across all sampling points, with higher stream velocities observed in spring ( $0.24 \pm 0.02 \text{ m s}^{-1}$ ) than in autumn ( $0.12 \pm 0.01 \text{ m s}^{-1}$ ) (Table 2; Table B2)."

"DO was higher in winter and spring than in summer and autumn ( $p < 0.001$ ).  $\text{NO}_3\text{-N}$  and TDN concentrations were highest in winter and lowest in autumn and summer ( $p < 0.01$ ), while  $\text{NH}_4\text{-N}$ , DOC, and DON showed no significant seasonal variation ( $p > 0.05$ ; Table 2; Table B2)."

L407 - either mention that the scales are not the same between the 3 columns, or preferably log transform the Y axis and use a consistent scale to facilitate comparisons between catchments.

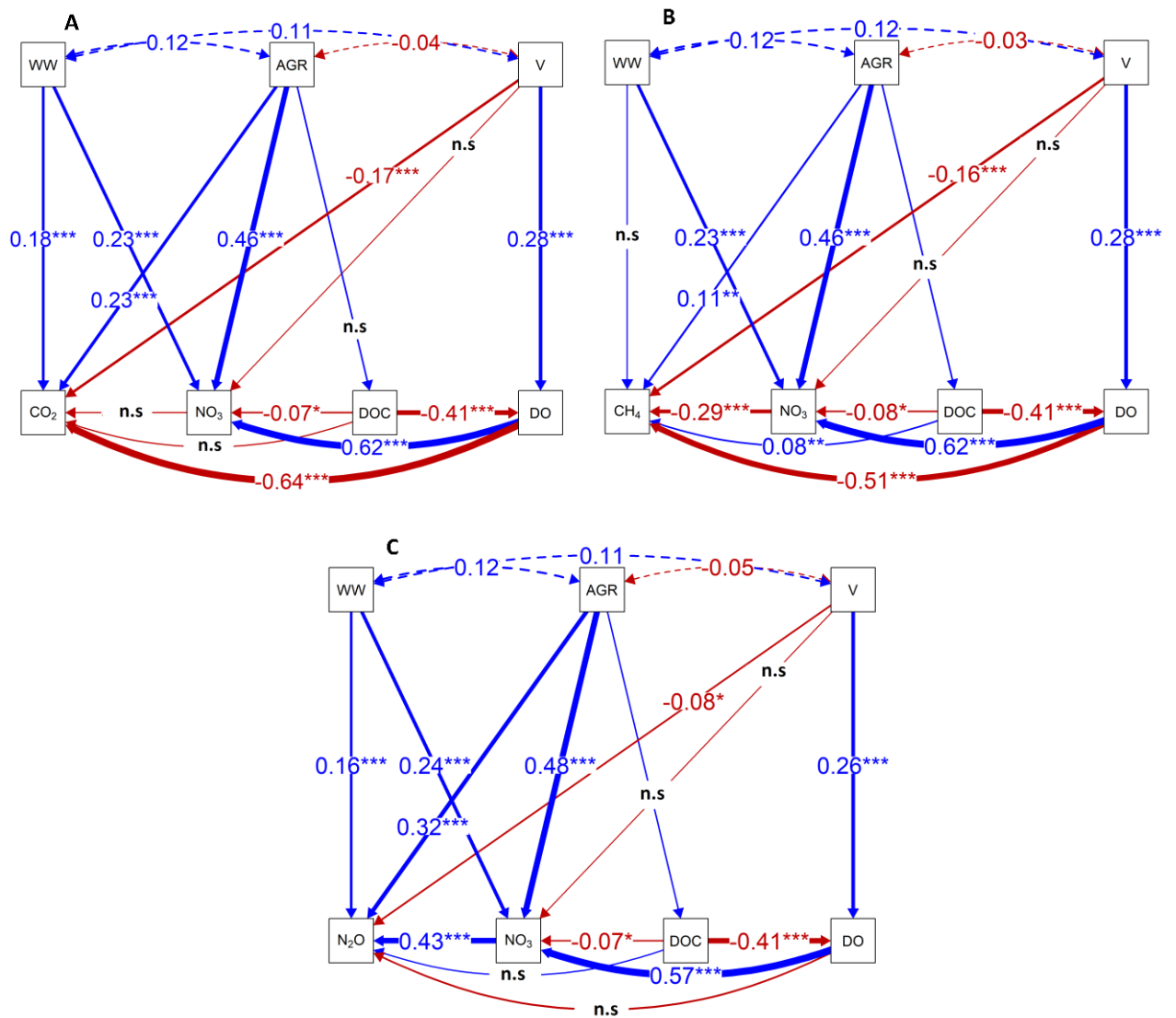
**Response:** Thank you for your critical observation and suggestion. We have redone the figure to make the scale more consistent.



L460 - the light blue and red numbers and lines are not easy to see, reconfigure the plot to make those darker or something.

**Response:** Thank you for your critical observation and suggestion. We have redone the figure to colors to be better visible.





L477 – It is

**Response:** Thank you for your critical observation. We have rephrased the statement to make it clearer.

"Overall, the annual CO<sub>2</sub>-equivalent emissions from anthropogenic-influenced streams (~71 kg CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup>) were up to 20 times higher than from natural forested streams (~3 kg CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup>; Fig. 6)."

L517 – seasons

**Response:** Redone

L598 - cite the relevant reviews and syntheses here

**Response:** Thank you for the suggestion. We have added three references with globally synthesized data in our comparison (Hu et al., 2016, Stanley et al., 2016; Li et al., 2021)

" This study's daily CH<sub>4</sub> and N<sub>2</sub>O diffusive flux ranges from both streams and ditches are mostly within the same order of magnitude as those previously reported in global synthesis studies (Table 3: Hu et al., 2016; Stanley et al., 2016). In contrast, this study reported among the highest fluvial CO<sub>2</sub> emissions compared to other regional and global studies, with significant mean fluxes of up to 51 g-C m<sup>-2</sup> d<sup>-1</sup> (Table 3)."