Reviewer 1

Review of the paper "Multidecadal trends in CO2 evasion and aquatic metabolism in a large temperate river" by An Truong Nguyen et al.

This paper is focused on a timely and relevant question, which is to better under how fluvial ecosystems regulate the global C cycle. The data set, with more than three decades of data, is unique not only because of its length but also because there are very few high temporal resolution data of this quality in large rivers. I sincerely congratulate the authors for their vision and perseverance to put together this impressive data set. Moreover, the paper reports interesting results illustrating that rivers can act either as sources or sinks of carbon, and that this pattern can change seasonally but also at large time scales depending on the nutrient status of the ecosystem. This finding has important implications for understanding how fluvial networks work and their contribution to global C fluxes under present and future anthropogenic pressures. Overall, I think this research will be of interest to the audience of Biogeoscience, though the paper requires major changes to improve clarity and streamline data analysis and the interpretation of the results before publication. Below, I provide some general and specific comments and suggestions, which I hope will be of help to the authors when crafting the revised version of the paper.

Response: We would like to thank the reviewer for these extensive comments and suggestions. We have addressed each of the general and specific comments below in this blue color font.

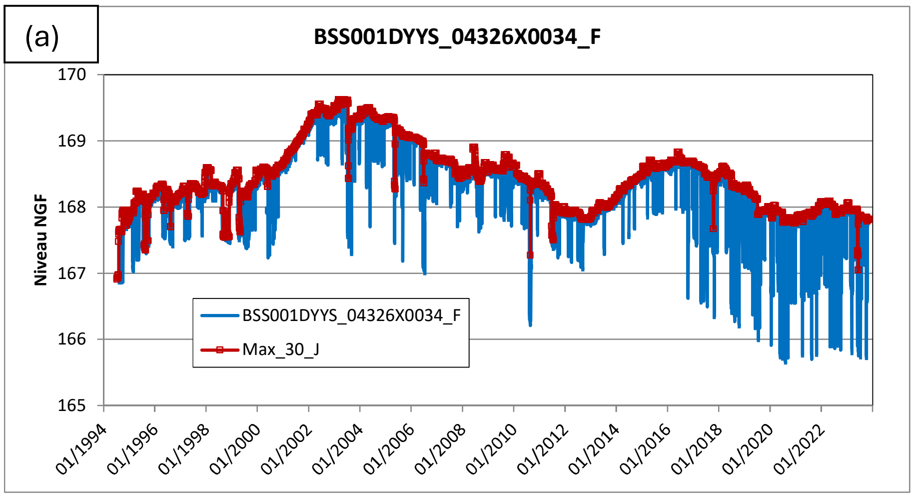
General comments

Long-term trends in groundwater CO2 inputs. One of my main concern is about long-term changes in groundwater CO2 inputs. As mentioned by the authors, it seems that the observed long-term decrease in FCO2 is mostly associated with a decrease of about 50% in groundwater inputs between the phytoplankton dominated and the macrophytes dominated periods. How reasonable this is? At the very end of the discussion, the authors suggest that there has been a generalized decrease in groundwater CO2 fluxes in the Loire catchment. Yet, it is not clear whether the long-term trend in discharge data support this explanation. How reasonable is to think that CO2 concentrations in groundwater have change if there have not been large changes in groundwater levels, neither in weathering rates. Overall, this flux is highly uncertain, and difficult to constrain with independent data.

Response: We agree that these external fluxes are uncertain and difficult to constrain with current data. First, we want to clarify that the observed decrease in our calculated external CO2 inputs is substantial. The mean annual external CO2 input decreased from 1008 ± 551 in the 1990-2000 period to 472 ± 129 gC m^{-2} yr^{-1} in the 2011-2021 period (Table 1), a reduction that exceeds the inter-annual variability and propagated uncertainty of our estimates. Therefore, we interpret this as a significant long-term shift that requires explanation.

To address question of "How reasonable is this?", we frame the argument as follows: a decrease in external CO2 flux must be driven by either (1a) a decrease in groundwater discharge (Qgw), (1b) a decrease in groundwater CO2 concentration (Cgw), or (1c) a combination of both.

Regarding groundwater discharge (Qgw**):** While our data show a modest long-term decline in river discharge (~13% over 32 years, Figure S8), we now present new evidence from a representative local borehole at Montifault (20 km from our site). After removing pumping effects with the EROS model (Thiéry, 2018), the data show a clear decreasing trend in the piezometric level of the nappe since the 1990s (New Figure S_groundwater_level). This evidence for decreasing groundwater levels, also noted at a regional scale (Binet et al., 2022; Baulon et al., 2022), supports a reduction in groundwater discharge (Qgw) contributing to the river. However, the modest scale of these hydrological changes suggests they are insufficient to be the sole driver of the >50% decrease in the calculated external CO2 flux.



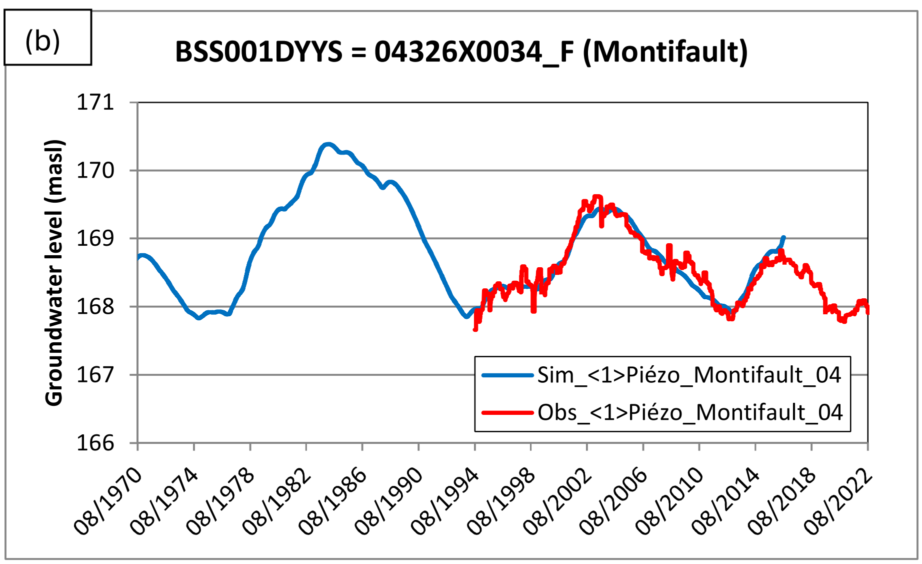


Figure S_groundwater_level: Decreasing trend in the groundwater level in Montifault (20 km from our site). (a) raw data, (b) after removing pumping effects with the EROS model (Thiéry, 2018)

Reference: Thiéry, D.: Logiciel ÉROS version 7.1. Guide d'utilisation, in: Rapport BRGM/RP-67704-FR, 175 pp., http://infoterre.brgm. fr/rapports/RP-67704-FR.pdf (last acccess: 12 October 2022), 2018.

Regarding groundwater CO2 concentration (Cgw): The discrepancy points towards a significant decrease in groundwater CO2 concentration as a key driver. To investigate this, we analyzed 30-year records of both pH and Total Alkalinity (TA) from several local groundwater monitoring stations near Dampierre (New Figure S_groundwater_quality). This analysis reveals a long-term increasing trend in groundwater pH of ~0.1-0.2 units while groundwater TA remained relatively stable, mirroring trends in the surface water (Figure 2a). At stable alkalinity, a pH increase of 0.1 units corresponds to a ~20-25% decrease in pCO2, lending more direct support to our hypothesis.

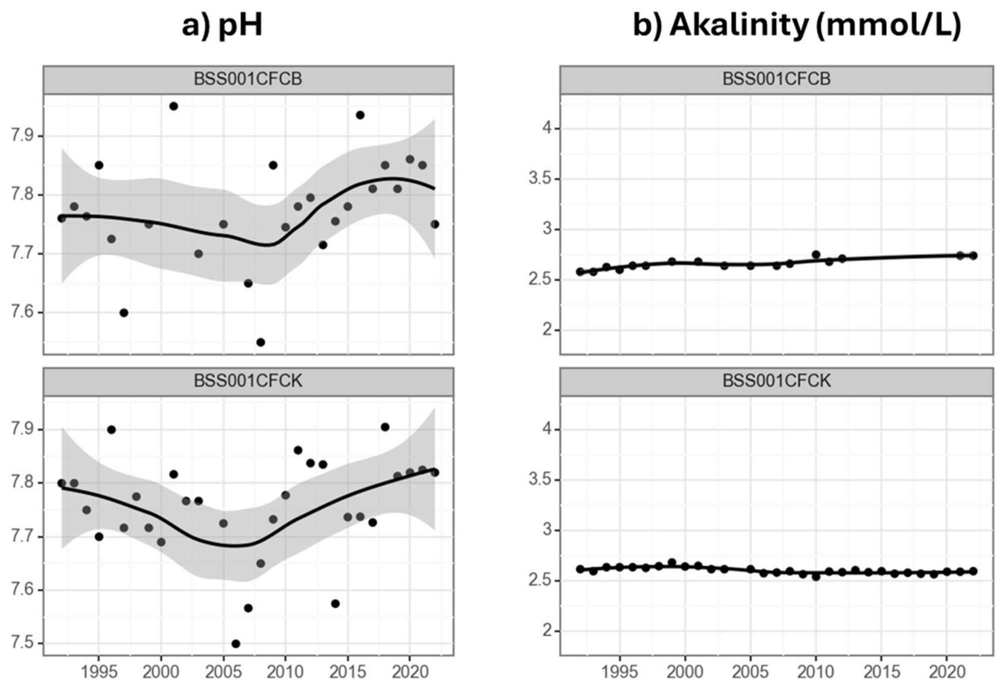


Figure S_groundwater_quality. pH and alkalinity in groundwater monitoring stations in the vicinity (5 km radius) of the Dampierre study site (1990-2021). Points represent individual measurements, and solid lines are LOESS smoothers with 95% confidence intervals (shaded areas). Source: https://hubeau.eaufrance.fr/page/api-qualite-nappes

We will emphasize this uncertainty more clearly in the Discussion and frame our interpretation as a hypothesis.

Re-oligotrophication. This phenomenon becomes crucial for understanding the temporal patterns in stream metabolic activity and CO2 sources, yet the magnitude of change of nutrients and DOM in the study river over time is barely mentioned. Even if this shift in water chemistry has been explained in a previous paper, some more quantitative information will help to better framed the discussion and interpretation of the results of this paper.

Response: We will add specific details on nutrient and chlorophyll-a changes, referencing previous work on the Loire River. We will also recall these changes in the Discussion when interpreting shifts in metabolic activity and CO2 sources. We will update the main manuscript

"The Loire River (France) was one of the most eutrophic in Europe at that time with total phosphorus (TP) concentrations frequently exceeded 0.2 mg P L-1 and chlorophyll-a concentrations often surpassed 100 µg L-1, with summer peaks reaching over 200 µg L-1 (Minaudo et al., 2015; Moatar & Meybeck, 2005). Following efforts to reduce nutrient inputs, the Loire River underwent a significant re-oligotrophication process. Between the early 1990s and the mid-2000s, TP concentrations declined by approximately 50-70%, and mean summer chlorophyll-a concentrations decreased from >100 µg L-1 to <30 µg L-1 (Minaudo et al., 2015; Diamond et al., 2022)."

Terminology. The authors use many different concepts to describe whether their system is dominated by macrophytes or phytoplankton, whether it is in an oligotrophic or eutrophic state, and finally classify the system behavior in four trophflux states as a function of CO_2 fluxes and metabolic activity, which is the cornerstone of the results. For instance, "macrophyte-dominated" and "oligotrophic" regimes as well as "phytoplankton-dominated" and "eutrophic" regimes are used at the beginning. Also, the authors refer to "regime", "states", or "periods" non-consistently when referring to either "trophic conditions" or to metabolic activity. Overall, my suggestion is to simplify a bit this terminology and make sure to refer always in the same terms to the same concepts. For instance, only use either macrophyte- vs phytoplankton-dominate OR oligotrophic- vs eutrophic- regimes, and be consistent referring to either "states", "regimes", or "periods".

Response: We agree with consistent terminology.

Revised: " ...coincident with the shift from a phytoplankton-dominated trophic regime (around 2005) and the subsequent shift in the stream's metabolic characteristics (around 2012)

Metabolic stoichiometry to convert O_2 to CO_2 moles. More details on these calculations are needed. An important aspect is whether conversions were similar for the phytoplankton- and the macrophyte-dominated periods, and to discuss the uncertainty associated with these calculations.

Response: In our metabolism calculations, gross primary production (GPP) and ecosystem respiration (ER) were initially estimated in O_2 units by the streamMetabolizer model. We converted these to carbon units (mmol C m2 d, as reported in the manuscript) using a molar O_2:C ratio of 1:1. For simplicity, we did not vary the photosynthetic or respiratory quotient between the phytoplankton-dominated and macrophyte dominated periods.

This approach is supported by recent study of Diamond et al. (2025; some of us are co-authors). Indeed, a detailed analysis by Diamond et al. (2025) on this dataset found a significant difference in the Ecosystem Quotient (EQ: apparent mol O_2 produced per mol DIC consumed), with a median EQ of ~1.3 during the phytoplankton-dominated period and ~1.0 under macrophyte dominance. They noted this ~30% change in EQ largely explains the observed change in O_2-based GPP between the two regimes, implying that GPP in carbon units was likely more stable over time than our 1:1 conversion would suggest. However, this measured difference in EQ is primarily relevant during autotrophic periods. The vast majority of the internal CO_2 production in our system occurs during heterotrophic periods (i.e., winter), where the Respiratory Quotient (RQ) is the key stoichiometric parameter. There is not sufficient evidence to suggest that RQ varied systematically over time during these dominant heterotrophic periods. Therefore, while using a 1:1 ratio likely underestimates the magnitude of carbon fixation (autotrophic sink) during the phytoplankton-dominated summers, we posit it remains a reasonable first-order approximation for the heterotrophic periods that dominate the internal CO_2 source budget. Based on this evidence, we consider the fixed ratio a conservative and justified assumption for estimating multi-decadal trends.

We will clarify this assumption in the methods.

Method: "GPP and ER were then converted to carbon units (g C m-2 d-1) using a fixed molar O_2:C ratio of 1:1. This assumption is widely used in river metabolism studies and reflects the stoichiometry of aerobic metabolism (Trentman et al., 2023). Although photosynthetic and respiratory quotients (PQ and RQ) can vary with autotrophic community composition, recent long-term analysis of the Loire River by Diamond et al. (2025) showed that such variability does not lead to cumulative bias in net ecosystem production or CO_2 budgets when integrated over decadal timescales. Therefore, we adopt this approach as a reasonable and conservative approximation for estimating long-term carbon dynamics, while acknowledging it as a source of short-term uncertainty."

Discussion: The interpretation of carbon-based metabolic rates is further nuanced by ecosystem-specific stoichiometry. While our study uses a consistent 1:1 O_2:C ratio for evaluating long-term trends, recent work by Diamond et al. (2025) on this dataset demonstrated that the median Ecosystem Quotient (EQ) shifted from ~1.3 during the phytoplankton period to ~1.0 during the macrophyte period. This EQ change, linked to differing autotrophic community C:N ratios, implies that C-based GPP was likely more stable across the decades than a simple 1:1 conversion of O_2 -GPP would suggest. This varying EQ primarily affects the interpretation of metabolic fluxes during autotrophic periods. However, the majority of the annual internal CO_2 production in the Loire occurs during heterotrophic winter periods, for which there is less evidence of a systematic, long-term change in the Respiratory Quotient (RQ). Thus, while our use of a 1:1 ratio may underestimate the magnitude of the historical autotrophic carbon sink, we consider it a reasonable approximation for assessing trends in the dominant heterotrophic internal CO_2 source.

Reference: Diamond, J. S., Nguyen, A. T., Abril, G., Bertuzzo, E., Chanudet, V., Lamouroux, R., & Moatar, F. (2025). Inorganic carbon dynamics and their relation to autotrophic community regime shift over three decades in a large, alkaline river. Limnology and Oceanography.

Comment: Change point analysis and statistical analysis. Is this analysis important enough to keep it in the main manuscript? At the end of the day, the authors are splitting the data set per decades. While I agree that the changepoint analysis somehow supports to split the data like by decades, I wonder whether it might be enough to add this analysis in the supplementary materials. On the other hand, the results would be better supported if the authors use statistical tests to explore whether differences among periods (and/or states) for the different variables are statistically significant. This would help to more clearly distinguish the most remarkable changes, and avoid qualitative statements.

Response: We believe the change point analysis is valuable for the main manuscript because it provides an objective basis for dividing the 32-year dataset into periods that reflect statistically identified shifts in key variables (FCO_2, NEP, etc.), rather than relying on arbitrary decadal splits or solely on previously published ecological shift years which might not perfectly align with the specific biogeochemical fluxes we are analyzing.

Regarding statistical testing, we agree with the reviewer and have now implemented statistical comparisons among periods within each trophic state. However, note that our manuscript also presents long-term trend analyses (Theil-Sen slopes) for annual values of key variables within each trophlux state over the entire 32-year duration (Figure 3). This addresses the gradual changes over time, not only for 3 decades.

We conducted non-parametric Kruskal-Wallis tests to assess overall differences between decades within each trophic state for key variables (FCO2, NEP, discharge, temperature, and occurrence frequency). For cases where significant differences were detected ($p<0.05$), we performed post-hoc Mann-Whitney U tests with Bonferroni correction to identify which specific decade pairs differed significantly.

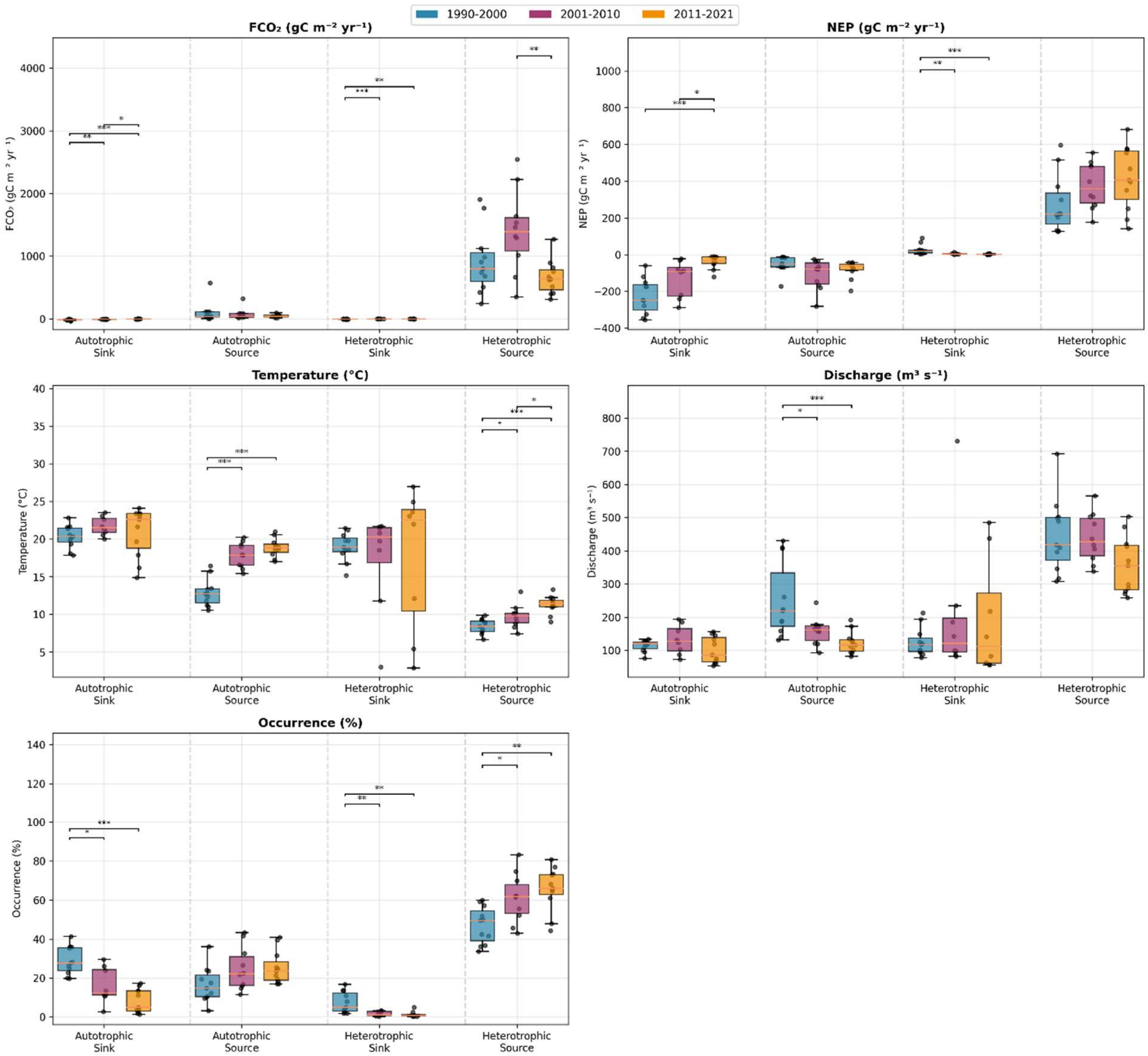


Figure S_Stats: Statistical comparison of key variables across decades (1990-2000, 2001-2010, 2011-2021) within each trophic state. Boxplots show median (horizontal line), interquartile range (box), and distribution of annual values (black dots). Colored boxes represent different decades: blue (1990-2000), purple (2001-2010), and orange (2011-2021). Statistical significance of differences between decades was assessed using Kruskal-Wallis tests, with significant pairwise differences (Mann-Whitney U test) indicated by horizontal bars with asterisks (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

Internal vs external sources of CO_2. While I understand the point of the authors, this is an ***oversimplification of CO2 sources***. For instance, by referring to “external CO_2 sources” the authors imply there is no other internal sources than aerobic metabolism producing CO_2 in the study system. How reasonable is to assume that there is **no anaerobic metabolism**? The authors should include some rational about this assumption, or else refer to “Other sources” rather than to “External sources”. Regarding “internal sources”, I wonder ***whether diel signals of dissolved oxygen fully capture the metabolism associated with photoautotrophs***. In Table 1, the authors report negative values for “external CO_2 inputs” which seems unrealistic. A potential explanation could be a systematic underestimation of the photoautotrophic activity by either phytoplankton or macrophytes, which might be more evident during this state, though may be happening also during other states. On the other hand, how feasible is that “external inputs” vary so much among states within a given decade? The authors should better discuss and, if possible, constrain, this factor to the best of their knowledge.

Response: We acknowledge that our terminology and framework are simplified but it is typical in many previous studies. In our analysis, “internal CO_2 source” specifically refers to CO_2 produced by net ecosystem respiration. We want to state clearly that we do not assume there is no anaerobic metabolism. The diel oxygen method for calculating ecosystem respiration (ER) implicitly accounts for most anaerobic metabolism. This is because the reduced secondary metabolites produced during anaerobic processes (e.g., NH_4^+, Mn^{2+}, Fe^{2+}, sulfides) are typically re-oxidized in other parts of the ecosystem, a process that consumes oxygen and is therefore captured within the integrated ER term. The only significant anaerobic pathways not accounted for by this oxygen consumption are denitrification (which produces N_2 gas) and methanogenesis. In a large, generally well-oxygenated river like the Loire, these pathways are expected to be a minor component of the overall carbon and oxygen budget compared to aerobic respiration. We will add a concise explanation of this to the Methods section to clarify that our ER term represents a robust measure of total ecosystem oxygen demand.

Regarding whether diel O_2 signals fully capture the metabolism associated with all photoautotrophs, our open-water diel O_2 method (*streamMetabolizer*) quantifies net changes in DO in the water column. This integrates the metabolic activity of phytoplankton suspended within the water and the portion of benthic photoautotroph (e.g., submerged macrophytes, benthic algae) metabolism that results in O_2 exchange with the overlying water. We agree there is a potential issue if macrophytes or benthic algal create very localized O_2 supersaturation that do not fully mix with the water on a diel timescale, then our method might slightly underestimate GPP.

We add a sentence in the Methods acknowledging this: “Diel DO measurements primarily reflect pelagic and well-mixed benthic metabolism; extremely localized autotrophic production (for example, within dense macrophyte stands) may not be fully captured, potentially leading to an underestimation of GPP during periods of high macrophyte activity”. However, given the size and flow of the Loire as a large temperate river, we expect the water to be sufficiently mixed that most macrophyte oxygen production is recorded.

Regarding negative external CO_2 in Table 1, particularly for the Heterotrophic-Sink state (which we note is rare, occurring 1-7% of days annually): Our manuscript already provides a physical explanation for this phenomenon. We state, 'The heterotrophic-sink state implies that despite the net conversion of biomass into water column CO_2, there is still a CO_2 undersaturation relative to the atmosphere, likely due to prior autotrophic uptake. We expect the heterotrophic-sink state to be a temporary occurrence, reflecting temporal lags...'. This reflects the river acting as a strong net sink for CO_2 from all sources combined due to this biologically-driven undersaturation, not an active consumption by external sources like groundwater.

We agree with the reviewer's suggestion that a systematic underestimation of photoautotrophic activity (GPP) could also contribute to these calculated negative values is a pertinent consideration. If true GPP were indeed higher, particularly in periods preceding or during the Heterotrophic-Sink state, the calculated NEP would be more positive (or less negative). This would, in turn, make the calculated 'External CO_2 ' less negative or potentially positive. While our diel O_2 method captures bulk water metabolism, and we expect considerable mixing in the Loire, some underestimation of GPP from dense macrophyte beds (due to direct O_2 loss or localized consumption without full mixing) is a possibility we will discuss more explicitly as a potential contributing factor in the supplement information methods.

Concerning the variability of calculated 'external inputs' among states within a decade: These are mean annual values for days falling into specific trophlux states. Different trophlux states are demonstrably associated with different mean hydroclimatic conditions (e.g., temperature, discharge, as shown in Table 1).

Sources of uncertainty. The authors need to better consider in their calculations the different sources of uncertainty. The supplementary materials tackle some of these sources of uncertainty, but some of this rationale needs to be moved to the main text, and other additional sources of uncertainty such as those associated with respiration and photosynthetic coefficients, anaerobic respiration, k600 in large streams (note that Raymond equations are useful for small streams with complete water column mixing, which is not the case of large rivers), and GPP not captured by DO signals in the water column (which may happen for macrophytes) should also be considered.

Response: Several sources of uncertainty mentioned have been addressed in detail in our responses to other comments and will be incorporated into the revised manuscript:

- O_2:C stoichiometry: we clarify our 1:1 O_2 :C assumption in the Methods and extensively discuss the implications of the system-specific varying Ecosystem Quotients (EQs of 1.3 and 1.0) found by Diamond et al. (2025) in our Discussion, contextualizing our C-based NEP and CO_2 source partitioning.
- k600 in large streams: we expand the Methods section to discuss the comparison of our streamMetabolizer-derived k600 with Raymond et al. (2012) equations, acknowledge the challenges of k600 estimation in large, potentially incompletely mixed rivers, and explain why discrepancies between methods might occur, particularly seasonally. We justify our choice of streamMetabolizer k600 for internal consistency.
- GPP not captured by DO signals (macrophytes): we will add a discussion on the potential for GPP underestimation in dense macrophyte beds due to mechanisms like direct O_2 loss via ebullition or localized O_2 dynamics not fully mixing with the bulk water.

Contribution of internal sources to total CO_2. Overall, I wonder whether it makes sense to report -NEP/FCO_2 in all cases since the implications of the mass balance are quite different depending on whether the stream is acting as a source or a sink of CO_2. In particular: (1) No doubt about what -NEP/FCO_2 implies for the heterotrophic-CO_2 source state; (2) For the autotrophic-CO_2 source state, the contribution of the stream to FCO_2 is actually 0%, and photoautrotrophs could contribute to reduce “external CO_2 inputs” by xx % (i.e. -NEP/external CO_2 rather than -NEP/FCO_2); (3) For the heterotrophic-CO_2 sink state, it has no sense to me that groundwater is not contributing CO_2, unless the stream is losing water, and in this case, there might be either an unaccounted pool fixing CO_2 from the water column, or GPP is systematically underestimated for whatever reason; (4) For the autotrophic-CO_2 sink state, the internal source could contribute to balance out 100% of the “external CO_2 sources”, and contribute to fix additional CO_2 from the atmosphere (i.e. not sure whether -NEP/FCO2 is really meaningful in this case). From a mass balance perspective, Figure 5 (in the discussion) makes much more sense than Table 1, and my feeling is that the manuscript would be more easy to follow if the results focus on the mass balances.

Response: Actually, the footnotes * and ** in Table already try to explain this, but it may still remain complex for readers. As also discussed in our response to your General Comment regarding the interpretation of 'External CO_2 ' and negative values, we will revise the presentation in Table 1 and the associated text to improve clarity and ensure academic soundness.

Regarding the Heterotrophic-Sink state: We maintain that this state is physically plausible and does not imply an absence of groundwater CO_2 contribution. This transient state is explained by the carbonate system's buffer capacity. A significant percentage of Heterotrophic-Sink occurrences directly follow Autotrophic-Sink periods (Diamond et al., 2025). The intense biological CO_2 drawdown during the preceding autotrophic phase creates a large CO_2 deficit. Due to the buffering delay, the river can remain a net sink for atmospheric CO_2 even as it briefly switches to net heterotrophy, before net respiration is sufficient to overcome the deficit and turn the river into a CO_2 source.

To improve the manuscript's clarity as suggested, we will implement the following changes:

- Revise Table 1: Only report the percentage for the Heterotrophic-Source state.
- We will explicitly state in the Methods that the –NEP/FCO2 ratio is used to quantify internal source contribution specifically for the Heterotrophic-Source state, and for all other states, the mass balance is presented in Figure 5

Specific comments

Comments from reviewer 1	Responses from Nguyen et al.,
Abstract L26-27. For (ii), not clear from this sentence whether the contribution of aquatic metabolism was higher or lower during the eutrophic or the oligotrophic trophic regime. For (iii), better highlight the predominant role of the river as a source of CO2. For (iv), might be more informative to highlight the seasonality of FCO2, and how this was modulated by the trophic regime rather than referring to the hysteresis patterns.	Revised (ii): "ii) the mean annual contribution of internal CO2 production (net ecosystem respiration) to total FCO2 was 40%, with this contribution generally increasing during the later oligotrophic period (from ~20-40% to ~60-75% in the heterotrophic-source state) as overall FCO2 decreased, though varying substantially by year;" Revised (iii): "iii) while the river predominantly acted as a CO2 source, it occasionally functioned as a CO2 sink (FCO2 < 0) during summer, particularly in the earlier eutrophic period (1990-2000), though this sink behavior constituted a minor component (-0.6%) of the overall multi-decadal FCO2 budget;" Revised (iv): " iv) FCO2 showed strong seasonality linked to discharge, exhibiting hysteresis where FCO2 levels at equivalent discharge were 1.5 to 2 times higher during the rising limb (autumn) compared to the falling limb (spring); the magnitude of this hysteresis diminished in the later oligotrophic period, indicating a changing seasonal discharge control."
L31."dynamic within and across years" as a function of what? The amount of nutrients?	Revised: "This study makes clear that river FCO2—and the source of this CO2—is highly dynamic within and across years, driven by hydro-climatic variations and biological activity, and that longer-term shifts in the river's trophic regime fundamentally alter the balance between internal and external CO2 sources."
Introduction L38. Better say "is assumed to come", since there are already several published studies challenging this assumption.	Revised: "A significant portion of CO2 flux (FCO2) emitted from rivers is often **assumed** to come from "external" sources..."
L54-56. In which way these variables controlled by discharge (inputs of carbon and nutrients) would influence metabolic activity and the balance between GPP and ER?	Revised: "...as changes in flow rates affect the transport of nutrients and organic carbon from the surrounding land. These inputs serve as substrates and resources for stream metabolism, influencing rates of GPP (via nutrient availability) and ER (via organic carbon supply), and thus the overall NEP balance. Discharge also modulates the physical exchange of CO2 between the river and the atmosphere (Cole et al., 2007; Hotchkiss et al., 2015)."

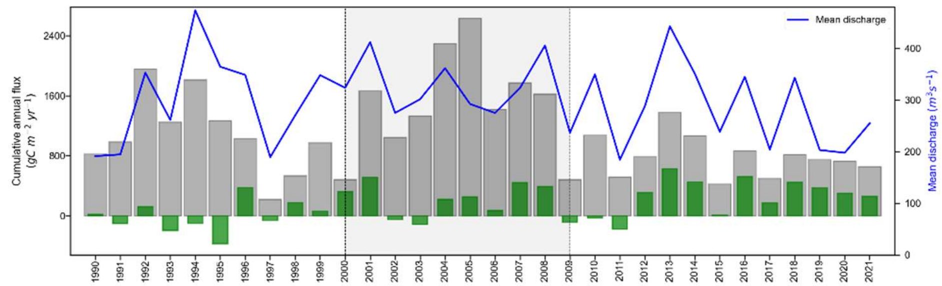
L72-74. Clarify what do you mean by "positive NEP yields local organic matter increases". Do you mean in the form of algal biomass? Increases in particulate and dissolved organic matter? Explain why "this autotrophic state" is most common in larger rivers, and clarify why "this is typically missed by FCO2 sampling campaigns".	Revised: "In this "autotrophic" state (GPP > ER), positive NEP means a net production of organic matter within the reach, leading to increases in biomass (e.g., algal, macrophyte) and potentially contributing to particulate and dissolved organic matter pools. Such autotrophic periods, sometimes leading to CO2 undersaturation, can be more prevalent or sustained in larger rivers due to factors like greater water residence times, increased light availability across wider channels, and potentially a greater buffering capacity against rapid changes in external CO2 inputs compared to smaller streams (Hotchkiss et al., 2015). However, these periods, especially if transient or occurring outside of typical low-flow summer conditions, can be missed by FCO2 sampling campaigns that are often infrequent or biased towards specific seasons, thus underestimating their occurrence and impact (Aho et al., 2021)."
L75. Indicate if this finding is general to all large rivers, or if it was observed in specific rivers.	We will revise as below
L81. For contextualization purposes, provide some information on how nutrient concentrations changed between the eutrophic and oligotrophic regimes and this phenomenon happen. It is also important to recall this in the discussion, to better interpret the large changes in both groundwater CO2 inputs and metabolic activity within the river.	The Loire River (France) was one of the most eutrophic in Europe at that time with total phosphorus (TP) concentrations frequently exceeded 0.2 mg P L-1 and chlorophyll-a concentrations often surpassed 100 µg L-1, with summer peaks reaching over 200 µg L-1 (Minaudo et al., 2015; Moatar & Meybeck, 2005) Following efforts to reduce nutrient inputs between the early 1990s and the mid-2000s, TP concentrations declined by approximately 50-70%, and mean summer chlorophyll-a concentrations decreased to <30 µg L-1 (Minaudo et al., 2015).
L85. By the growing season do you mean spring and summer?	Revised: "...associated with reductions in GPP and NEP during spring and summer (Diamond et al., 2022)."
L83-87. These two sentences can be merged and shorten.	Revised: " This ecosystem transition was followed by a delayed shift in the river's metabolic regime around 2012–2014, with GPP declining and NEP decreasing by roughly 10% during the spring–summer growing season (Diamond et al., 2022)
L88. Please, could you provide other examples of re-oligotrophication in developed countries? This shift towards lower nutrient concentration in large rivers is not so evident giving the modest improvements in water chemistry observed in the last decades in Europe.	There are few well-documented examples of nutrient load reductions leading to ecological shifts in major rivers within developed countries. Our manuscript cites Ibáñez et al. (2023) for a global review of re-oligotrophication and its effects, which further supports this broader context.

	The Rhine River was with orthophosphate concentrations declining from >0.4 mg/L (early 1970s) to <0.1 mg/L (1998), accompanied by broader ecological rehabilitation efforts. Recent work on the Upper Mississippi River (USA) documented substantial macrophyte community recovery (1998–2020), including increased species diversity and shifts from free-floating to submerged plants. The River Thames (UK) exemplifies this trend, with phosphorus loads reduced by approximately 80% over 40 years due to improved wastewater treatment and agricultural management, though nutrient concentrations remain above ecological limiting levels and rising temperatures continue to promote algal blooms. Jarvie, H. P., Worrall, F., Burt, T. P., & Howden, N. J. K. (2025). A 150-year river water quality record shows reductions in phosphorus loads but not in algal growth potential. Communications Earth & Environment, 6(1), 62 Ibáñez, C., et al. (2022). Ecosystem-level effects of re-oligotrophication and N:P imbalances in rivers and estuaries on a global scale. Global Change Biology, 29(2), 261–282 Larson, D., Jones, M., Weigel, B., Gray, B., & Ovaskainen, O. (2024). River re-oligotrophication and hydrologic changes abruptly contributed to macrophyte community shifts and recovery (https://aslo.secure-platform.com/2024/gallery/rounds/16/details/11174)
L91-93. Note that the hypothesis is lacking the reasoning behind. Why did you expect an increase in the contribution of FCO2 from aquatic metabolism if, according to the earlier paragraph, you actually observed a decrease in NEP with reoligotrophication?	The reasoning was that reduced GPP would mean less CO2 uptake, potentially leading to higher pCO2 and thus higher FCO2. If ER remained similar or increased, then –NEP would increase, further boosting FCO2. The phrasing "internal source contribution would increase" refers to the ratio –NEP/FCO2. If –NEP increases (more net respiration) and FCO2 also increases or changes less drastically, the ratio can increase. Revised: "In this study, we used a 32-year daily dataset of coupled stream metabolism (NEP) and FCO2 to assess the temporal internal/external CO2 source contributions in the Loire River. With re-oligotrophication leading to observed decreases in GPP and NEP (Diamond et al., 2022), we hypothesized

	that: (1) overall FCO2 from the river would increase due to reduced CO2 uptake by primary production, and (2) the relative contribution of internal CO2 production (from net ecosystem respiration, –NEP) to this total FCO2 (i.e., the –NEP/FCO2 ratio) would also increase, as the system becomes effectively more heterotrophic or less autotrophic."
L94. Note that in the earlier paragraphs you refer to "regime" when talking about the trophic conditions, but to "states or periods" when referring to stream metabolic activity. To help the reader, better be consistent with the terminology throughout.	We will check the entire manuscript to ensure we consistently Revised: " ...coincident with the shift from a phytoplankton-dominated trophic regime (around 2005) and the subsequent shift in the stream's metabolic characteristics (around 2012)
L96. How do you expect discharge to influence FCO2 in the first term? Would FCO2 increase or decrease with discharge? Why will Q influence FCO2 differently in the macrophyte dominated period across seasons? And why this seasonal influence will not emerge in the phytoplankton dominated period?	Generally, FCO2 is expected to increase with discharge due to increased turbulence (higher kCO2) and potentially increased delivery of CO2-rich terrestrial/groundwater (higher pCO2). We will include it in introduction: Revised: "Finally, discharge (Q) influences FCO2 through multiple mechanisms, including gas transfer velocity, delivery of external CO2, and inhibition of in-stream primary production. Phytoplankton GPP can be sensitive to Q (e.g., washout, turbidity), while macrophyte GPP may be less directly flow-dependent (Diamond et al., 2022). We therefore predicted that the overall control of Q on FCO2 would change with the shift to macrophyte dominance. Specifically, we anticipated that the seasonal hysteresis patterns observed in the FCO2-Q relationship (where FCO2 differs between rising and falling limbs of the hydrograph at similar Q) would be altered, potentially becoming less pronounced or showing a different shape if macrophyte GPP imparts a more stable baseline of CO2 uptake across varying flow conditions compared to phytoplankton."
L112. And during winters?	Revised: "During summer low flows (<150 m3/s), the study site is typically shallow (around 1 m deep) and wide (330 m); during winter, with higher discharges (e.g., >500 m/s), depths can increase significantly, typically ranging from 2 to 3 m."
L130. How did you transform stream metabolic rates from O2 to C units? Which stoichiometric ratios did you use (i.e., photosynthetic and respiration coefficients). Did you consider whether these coefficients vary between the phytoplankton- and the macrophyte-dominated regimes?	We now clarify this in the Methods where we discuss metabolism calculations Method: "GPP and ER were then converted to carbon units (g C m-2 d-1) using a fixed molar O2:C ratio of 1:1. This assumption is widely used in river metabolism studies and reflects the stoichiometry of aerobic metabolism (Trentman et al., 2023). Although photosynthetic and respiratory quotients (PQ

	and RQ) can vary with autotrophic community composition, recent long-term analysis of the Loire River by Diamond et al. (2025) showed that such variability does not lead to cumulative bias in net ecosystem production or CO2 budgets when integrated over decadal timescales. Therefore, we adopt this approach as a reasonable and conservative approximation for estimating long-term carbon dynamics, while acknowledging it as a source of short-term uncertainty.”
L137. Only 12% of discards is a big success! Why did you choose to fill the gaps? Please, indicate in the main text whether main results and conclusions hold if not filling the gaps.	The SI (Section S3) discusses the handling of physically impossible results from streamMetabolizer, showing that replacing with 75th percentile vs. setting to zero has minor impacts on annual totals (avg 1.3% for GPP). We chose to fill gaps to have a continuous daily time series for FCO2 and NEP calculations, which is required for accumulative annual budgets and consistent change point/trend analysis.
L139. I think Supplementary Section S2 is missing or, if it comes latter, Supplementary sections should be reordered to be cited in order in the main text.	We will check all SI citations for correct numbering and order.
L150-151. What do you mean by “river CO2 state compared over 32 years”? Do you mean that there were no long-term trends in concentration? That there were no statistically significant differences in average CO2 concentration between the two trophic regimes? Best used past tense.	Ok, we will clarify and use past tense. Revised: "...however, this uncertainty in TA did not significantly alter the classification of the river's CO2 status over the 32-year period when comparing pCO2 estimates using the mean TA versus TA at the bounds of its uncertainty range (Section S4, Table S1 in supplement)."
L156. Delete “with” after “multiplying”.	Revised: "The k600 was calculated by multiplying river depth with K600..."
L157. Add “the” between “with” and “seven”.	Revised: "...compared k600 with the seven fitted equations..."
L161-165. To be formal, define all the terms included in these equation (depth and T).	Revised: "Where: FCO2 is the CO2 flux (mmol C m-2 d-1), kCO2 is the CO2 gas transfer velocity (m d-1), CO2,water is the aqueous CO2 concentration in water (mmol m-3), CO2,air is the aqueous CO2 concentration in equilibrium with the atmosphere (mmol m-3), 'depth' is the mean daily river depth (m), K600 is the gas exchange rate coefficient normalized to a Schmidt number of 600 (d-1) from streamMetabolizer, ScCO2 is the Schmidt number for CO2 (unitless), and T is the water temperature in degrees Celsius (oC)."

L166.This subtitle is sort of funky. Something like “The trophlux categories” would be enough.	"Trophlux" is a term we introduced, combining "trophic" and "flux". We can make the subtitle more standard if preferred, like “Trophlux state definitions”
L167-169. Well, for NEP these states have been defined for decades. I think Odum reserves credit here.	Revised: "Following Odum (1956), if NEP is positive (GPP > ER), the river reach is considered net autotrophic, while if NEP is negative (GPP < ER), it is considered net heterotrophic." Odum, H. T. (1956). Primary production in flowing waters. Limnology and Oceanography.
L178. Could also the heterotrophic-sink state also occur during high discharges because of high gas exchange with the atmosphere?	In our 32-year dataset, we observed heterotrophic-sink states only during brief transition periods (1-14 days, typically June-August) when the system was shifting from autotrophic-sink to heterotrophic-sink under low discharge conditions, not during high flow events. We attribute the heterotrophic-sink state to a delay for heterotrophy compensate for the CO_2 depletion due to buffer capacity of the carbonate system in the alkaline waters. The physical constraints of mass balance make sustained heterotrophic-sink conditions during high discharge highly improbable. Besides, the gas exchange intensity will not change the direction of the CO_2 flux, only its intensity.
L191. Explain briefly what is a seasonal decomposed time series.	Revised: "...applied to seasonally decomposed time series of daily FCO_2 and NEP. This decomposition, performed using the statsmodels Python package (Seabold & Perktold, 2010), separates a time series into trend, seasonal, and residual components, allowing us to specifically identify change points in the characteristics of the seasonal cycle (e.g., amplitude changes) independently of the long-term trend.".
L198-201. For the reader to follow this prediction the expectations need to be better explained in the last paragraph of the introduction.	The revised introduction in your comment L96 addressed this
L201-204. Please, provide some hints of how the two metrics used to characterize the hysteresis loops are expected to change between the phytoplankton vs macrophyte-dominated regimes.	Revised: "We evaluated hysteresis loops by their direction (clockwise or counterclockwise) and magnitude (e.g., the difference in FCO_2 at equivalent discharge between the rising autumn limb and falling spring limb of the hydrograph). We anticipated that the shift to a macrophyte-dominated system, with potentially more stable GPP across flow conditions, might lead to a reduction in the magnitude (i.e., a "flattening") of these hysteresis loops for FCO_2 and NEP."

Results L210. Explain better this successive seasonal transition between autotrophic/heterotrophic of NEP, sink/source of FCO2.	Revised: "The pronounced seasonal variability drove successive transitions among different trophlux states. Typically, the river would shift from being heterotrophic and a CO2 source in winter, towards becoming more autotrophic in spring and potentially a CO2 sink in summer during low flows, before returning to heterotrophic source conditions in autumn with rising flows. This general seasonal pattern, involving changes in both NEP (autotrophic/heterotrophic balance) and FCO2 (sink/source status), recurred each year, though its specific timing and intensity varied (Figure 1c)."
L218-221. Would be helpful to include discharge in Figure 1.	We will consider adding a mean annual discharge to Figure 1 
L222. Change "-" by "to" between "-383" and "584" to smooth the reading (suggestion holds for the whole results section). Note that the units are expressed differently in the main text and in Figure 1 (y vs yr)	Revised: "...and NEP from -383 to 584 g C m-2 yr-1." We will correct "y-1" to "yr-1" throughout the manuscript and figures for consistency.
L224. Add "net" before "source".	Revised: "...the Loire River was a net source of CO2 to the atmosphere..."
Figure 2. Honestly, pCO2, pH, alkalinity, and k600 could be moved to the supplementary.	We feel these parameters are central to understand the timing of shifts we are discussing. Moving them to SI would detach the visual evidence for the identified change points from the main narrative. We prefer to keep Figure 2 in the main text but will ensure the discussion of these change points is focused and directly relevant to the main hypotheses.

L244-249. Better apply a statistical test for comparing average values for each period and variable.	Done
L250. For the whole section and throughout the manuscript, would be more helpful to the reader if you refer to “heterotrophic-CO2 source” rather than to “heterotrophic-source”. Also, the text would flow better if you refer consistently to the 4 trophlux states throughout.	We explained this in 2.3.3. Categorizing NEP-FCO2 states by autotrophic/heterotrophic and source/sink states
L255. Clarify whether this is the annual range or an average value for each decade, and if the later, provide s.e.	Revised: "The mean annual occurrence of the heterotrophic-source state varied across the decades, ranging from 47.3 ± 9.4% in 1990-2000 to 65.8 ± 11.3% in 2011-2021 (Table 1)..."
L256. “coinciding with low water temperature and high discharge”. This result is quite rough. Please use a two-way ANOVA test or similar to support this statement. Clarify whether the 90% refers to all data or to each decade.	Done, (New Figure S_Stats)
L257-259. Bis as my earlier comment. If these are averages per decades, add the s.e., and clarify to which decade refer each number.	Revised: "Within this heterotrophic-source state, the contribution of internal sources (–NEP/FCO2) to total CO2 emissions varied across the decades: it was 37 ± 27% in 1990-2000, 28 ± 9% in 2001-2010, and increased to 57 ± 10% in 2011-2021 (Table 1). This implies that external CO2 sources accounted for the remaining proportion in each period."
L260-261. This sentence is confusing. Moreover, two of the remaining three trophlux states act as sinks rather than sources of CO2, so why one would expect them to contribute to FCO2?	Revised: "The other three trophlux states (autotrophic-source, autotrophic-sink, and heterotrophic-sink) had a combined net impact of less than 10% on the total annual FCO2 budget, despite their substantial occurrence at times (Table 1). The autotrophic-source state made a small positive contribution to FCO2, while the sink states (autotrophic-sink, heterotrophic-sink) made small negative contributions (i.e., reduced the total net annual FCO2)."
L261-263. Just refer to the occurrence of the autotrophic-CO2 sink state to follow the same logic throughout.	Revised: "The autotrophic-sink state, driven by high GPP, typically occurred for 1–3 months during the summer growing season..."
L263. Are these average values? Then add s.e.	Revised: "The autotrophic-sink state reduced the net annual FCO2 budget by 3.0 ± 4.2% during 1990–2000 and by 0.4 ± 0.3% in 2011–2021 (Table 1)."

<table>
<tr><td></td><td></td></tr>
<tr><td>L267. Say more clearly that this state represented a small sink of CO2.</td><td>Revised: "...and made a minor negative contribution to the annual FCO2 budget (reducing it by 0.1% to 0.8% across the decades, Table 1)."</td></tr>
<tr><td>

Table 1. The caption should better explain the variables to make sure that the table is self-explanatory. All the “footnotes” included at the bottom of the table include important information to interpret the data and thus should be included in M&M (some of this info would help to answer some of the below questions). It would be helpful to provide statistical tests among periods and states, and rewrite the results of this subsection in light of the result of these statistical tests. This Table arises some issues on how the calculated variables should be interpreted. It would be nice to add some text in the M&M helping the reader to interpret these values. Another possibility is to focus on the mass balance calculations (now in the discussion). More specifically:

1. How can external CO2 sources be negative? This suggests that there is some other unaccounted process fixing CO2, and also uncertainties associated with your calculations, which should be better constrained. Overall is unrealistic to think that groundwater is not supplying, but consuming CO2.
2. How can be the contribution of internal processes to total CO2 evasion (i.e. -NEP/CO2) a negative value as reported for both the autotrophic-CO2 source? In this case, I would say that the contribution of internal processes to CO2 evasion is 0%, and that photoautotrophic organisms are contributing to fix more CO2 than supplied by groundwater.
3. How can the contribution of internal processes to total CO2 evasion be higher than 100% as reported for the autotrophic-CO2 sink state? In this case, are photoautotrophs fixing CO2 from the atmosphere to fulfill their photosynthetic requirements?.

</td><td>

Most of these questions are related to previous responses. We briefly answer here:

1. We will revise the Methods to state explicitly that Heterotrophic-Sink is an accounting outcome under specific transient conditions and does not imply a physical negative external CO2 source.
2. You are correct, the negative ratio is mathematically correct but confusing, we will remove this metric.
3. We will use the mass balance diagram in Figure 5 to clearly illustrate that NEP is the largest flux, supported by both FCO 2 and external inputs in the autotrophic-CO2 sink state.

Besides, in Table 2, the external CO2 is not negative, what is negative is the difference in the external flux between autumn and spring, ie this variable is higher in spring than in autumn. This is why we called it as hysteresis in the Table as below.

<table>
<tr><th></th><th>Flux*</th><th>1990–2000</th><th>2001–2010</th><th>2011–2021</th></tr>
<tr><td rowspan="3">River metabolism (g C m^{-2} d^{-1})</td><td>–NEP_{300} spring</td><td>-0.28 ± 0.41</td><td>-0.69 ± 0.11</td><td>0.83 ± 0.18</td></tr>
<tr><td>–NEP_{300} autumn</td><td>0.66 ± 0.15</td><td>1.43 ± 0.13</td><td>1.5 ± 0.06</td></tr>
<tr><td>–NEP_{300} hysteresis</td><td>0.94 ± 0.43</td><td>2.12 ± 0.17</td><td>0.68 ± 0.18</td></tr>
<tr><td rowspan="3">Total CO_2 fluxes (g C m^{-2} d^{-1})</td><td>$FCO_{2\ 300}$ spring</td><td>1.55 ± 0.44</td><td>2.65 ± 0.15</td><td>2.42 ± 0.12</td></tr>
<tr><td>$FCO_{2\ 300}$ autumn</td><td>4.77 ± 0.14</td><td>4.15 ± 0.25</td><td>2.34 ± 0.08</td></tr>
<tr><td>$FCO_{2\ 300}$ hysteresis</td><td>3.22 ± 0.47</td><td>1.5 ± 0.29</td><td>-0.09 ± 0.14</td></tr>
<tr><td rowspan="3">External CO_2 flux (g C m^{-2} d^{-1})</td><td>($FCO_{2\ 300}$+ NEP_{300}) spring</td><td>1.83 ± 0.2</td><td>3.34 ± 0.05</td><td>1.59 ± 0.14</td></tr>
<tr><td>($FCO_{2\ 300}$+NEP_{300}) autumn</td><td>4.12 ± 0.05</td><td>2.72 ± 0.12</td><td>0.83 ± 0.05</td></tr>
<tr><td>($FCO_{2\ 300}$+NEP_{300}) hysteresis</td><td>2.29 ± 0.2*</td><td>-0.62 ± 0.13**</td><td>-0.76 ± 0.15</td></tr>
<tr><td rowspan="3">–NEP/FCO_2 (%)</td><td>–NEP/$FCO_{2\ 300}$ spring</td><td>-26 ± 33%</td><td>-26 ± 5%</td><td>34 ± 6%</td></tr>
<tr><td>–NEP/$FCO_{2\ 300}$ autumn</td><td>14 ± 3%</td><td>34 ± 1%</td><td>64 ± 1%</td></tr>
<tr><td>–NEP/$FCO_{2\ 300}$ hysteresis</td><td>40 ± 34%</td><td>61 ± 5%</td><td>30 ± 6%</td></tr>
</table>

* The hysteresis flux is equal to the difference of flux in autumn and spring, i.e., 2.29 = 4.12–1.83, where the 300 subscript refers to the fact that these measurements are averages from mean discharge at 300 m^3 s^{-1}.
** Negative hysteresis flux indicates lower flux in autumn (rising waters stage), i.e., -0.62 = 2.72–3.34

</td></tr>
</table>

L283. Better fully write down what -13%/12 years mean, since this is an important result. The same in line 289.	This is a total reduction of approximately 13% over the 32-year study period
L292. Better write "with either annual discharge or annual temperature".	Revised: "The decrease in +NEP was not significantly correlated with either annual discharge ($R2 = 0.03$) or annual temperature ($R2 = 0.00$) (Table S2)."
L292. What about the other two states?	We will mention the trends for the other two states as well for completeness.
L276-281. So, if there is a 62% reduction in external inputs, but only a 13% reduction in discharge, what could explain the reduction in groundwater CO2 concentration over time?	We discussed this in the response in the previous comments. Basically, the decline in external CO2 likely reflects a decrease in CO2 concentration in groundwater inflows or less connectivity.
Figure 3. Why did you use Theil-Sen slopes rather than regular linear slopes? Mention this briefly in M&M. Add "only for the heterotrophic-CO2 source state" in f.	We indeed explained it in Methods that we used Theil-Sen slope from Mann-Kendall because it's robust to outliers and not assuming normal distribution, referencing pyMannKendal
L301. The M&M methods should better explain how the seasonality is imbedded in the rising and falling stages of the discharge.	Actually, the Methods (Lines 198-204) describe evaluating hysteresis against discharge across periods. The rising stage is autumn, falling is spring. This is standard hydrograph analysis. We will clarify more: Revised: "We specifically compared fluxes during the rising limb of the annual hydrograph (typically autumn, as discharge increases) with fluxes during the falling limb (typically spring) at equivalent discharge rates to quantify hysteresis."
L305- "and from CO2 sink to source". Really? It seems the stream was acting as a source almost all year in Figure 4, except for some particular days.	We double-checked Figure 4: indeed, in the early years (1990–2000) there were brief periods (particularly July–August) where FCO2 dipped below zero (river acting as a sink). That is why we described a seasonal sink phase in summer

L309. "The contribution of external sources largely mirrored these patterns" This sentence needs some extra clarification.	Revised: "The calculated contribution of external CO_2 sources (FCO_2 + NEP) also exhibited a clockwise hysteresis loop with discharge, with higher external contributions during the rising limb (autumn/winter) and lower contributions during the falling limb (spring) at equivalent discharge rates, generally mirroring the patterns observed for FCO_2 and –NEP (Figure 4, bottom row)."
Figure 4. Why are you showing temperature in the color ramp?	We included temperature as the color gradient in Figure 4 because temperature strongly influences both biological activity and gas exchange. Plotting temperature along the hysteresis loops allows the reader to see the seasonal progression (e.g., moving from cool conditions to warm and back) associated with the rising vs. falling limb. This additional context enriches the interpretation of the loops, as it aligns with seasonal metabolic drivers.
L316. Add "the" between "in" and "three".	Revised: "...varied across the three decades..."
L317-328. Is this analysis of the hysteresis at 300 m3/s really a fundamental result of the paper? I understand the authors are doing this analysis to showcase the seasonal patterns exhibited by the variables studied. But this is already shown in Figure 4. My suggestion would be to withdraw these text (and Table) from the results and just select some of these numbers to illustrate the magnitude of these seasonal changes in the discussion.	Q = 300 m^3/s is the yearly average discharge in Loire river. Figure 4 shows the overall loops, but extracting precise differences at a common discharge point from the figure is difficult. We believe Table 2 provides valuable quantitative support for the changes in hysteresis described. It's a concise table. We prefer to keep it to substantiate the claims about changing hysteresis magnitude.
L319. I don't think the lack of slope makes the relationship more linear or more predictable than in the previous two decades.	We rephrase to be more precise Revised: "The near-zero FCO_2 hysteresis magnitude at 300 m^3/s in 2011–2021 (Table 2) indicates that FCO_2 values at this discharge were very similar during both the rising (autumn) and falling (spring) limbs of the hydrograph. This reduction in the hysteresis loop area suggests that the relationship between FCO_2 and discharge became less dependent on the seasonal progression of the hydrograph in recent years, making FCO_2 at a given moderate discharge more predictable regardless of season."
Discussion L330-342. In this first paragraph, it might be good to put some numbers to this "re-oligotrophication process".	Addressed in General Comment

L336. How do the authors explain such a decrease in external CO2 sources? Is because decrease in groundwater discharge, CO2 concentrations, or both? This comes very late in the discussion, but may be good to provide some hint here.	Addressed in General Comment
L331. Add “contribution of” before “internal source”.	Revised: "...increase in the contribution of internal sources to FCO2..."
L340-342. The reason why the authors expected to observed a weaker discharge control on FCO2 when macrophytes dominated is not clear in the introduction, so it is difficult to follow the rationale here. Not clear either what the authors mean by “weakened discharge-external CO2 source”.	We mean that the relationship between river discharge and the magnitude of external CO2 inputs became less strong in the later period. If external source hysteresis flattened (Fig 4), it means external CO2 inputs became less variable between seasons at similar discharges.
L347-349. Can you be more specific on how climate or environmental changes influence the occurrence of trophlux transitions?	Revised: "This finding challenges the conventional understanding of large rivers as persistent CO2 sources and demonstrates how ecosystem metabolism can fundamentally alter carbon cycling patterns. The frequency of CO2 sink conditions observed in the Loire River, particularly during its earlier eutrophic period, reveals an important but often overlooked aspect of river carbon budgets. The transitions among these trophlux states are influenced by seasonal climatic drivers (temperature, solar radiation, discharge patterns, which themselves are subject to long-term climate change) and broader environmental changes like nutrient loading (e.g., re-oligotrophication), making such long-term analyses critical for systems undergoing similar pressures."
L354. Be consistent with terminology throughout. Delete “metabolic”, the trophlux state does depend on the metabolic regime by definition (bis in line 356). Not clear what these ranges in parentheses are referring to, is this a range for the three decades? Perhaps it would be easier to provide an average value of the annual occurrence for the 32 years.	Revised: "...the mean annual occurrence of the autotrophic-sink state depended strongly on the prevailing long-term trophic conditions of the river, decreasing from 28.7 ± 7.0% of days in the eutrophic 1990-2000 period to 7.3 ± 5.7% in the oligotrophic 2011-2021 period (Table 1). This decline was mirrored by an increasing decadal occurrence of the heterotrophic-source state (from 47.3 ± 9.4% to 65.8 ± 11.3% respectively, Table 1)."
L359-363. Has this oligotrophication process being accompanied by changes in DOM? Why a decrease in nutrient availability has influenced GPP more than ER?	Diamond et al. (2022) discuss these GPP/ER dynamics for the Loire. In our revised MS, we will add a brief explanation.

	Revised: "This shift in trophlux state dominance reflects the ecosystem's response to re-oligotrophication. Reduced nutrient availability, particularly phosphorus, directly curbed primary production (GPP) more substantially than ecosystem respiration (ER) (Diamond et al., 2022). ER is supported by both autochthonous organic matter (linked to GPP) and allochthonous inputs from the catchment, the latter of which may not have declined to the same extent or could have different controlling factors. Changes in dissolved organic matter (DOM) quantity and quality likely also accompanied this transition (Diamond et al., 2022). Consequently, the balance shifted towards greater net heterotrophy.
L370. Actually, more than a data point! You could say, for instance, "This study sheds new light" or "it's a relevant contribution"….	Revised: "This work provides a significant long-term perspective on the contribution..."
L372. Not sure "rigorously" is the best adjective in this case, something like "quantify at high temporal resolution" may be more appropriate.	Revised: "...the capacity to quantify the relative strength of these two fluxes at high temporal resolution..."
L379. "the temporal evolution of discharge is equally important to its magnitude"? Clarify, please.	Revised: "While the magnitude of discharge is a known control on CO2 dynamics, our results highlight that the seasonal timing and the rising or falling limb of the hydrograph are equally crucial for determining CO2 fluxes and sources, due to hysteresis effects."
L403. Change "had an annual CO2 sink" by "was acting as a CO2 sink"	"...the Loire River exhibited a net annual CO2 sink (i.e., cumulative annual FCO2 < 0) in almost half of the years studied within the 1990-2000 decade, driven by high rates of GPP."
L410. Why large river autotrophs benefit from being less affected by external CO2 sources?	Revised: "In addition, autotrophy can exert a stronger control on pCO2 dynamics in larger rivers compared to small streams. This is due to factors such as increased light penetration across wider channels supporting higher areal GPP, and a larger water volume where internal metabolic signals may be less rapidly overwhelmed by the proportional influence of external CO2 inputs from groundwater or riparian zones (Hotchkiss et al., 2015)."
L411. Well, if it occurred in 2005, it was not a "long-term shift", but an "abrupt shift".	The shift from phytoplankton to macrophyte dominance was a process that occurred around 2005, not instantaneously in that single year. Minaudo et al. (2015) describe this transition. "Long-term shift" here refers to the change in the dominant primary producer type over the multi-decadal study.

L415. Provide in parenthesis the rate of annual increase in temperature and of decrease in discharge to get a sense of the magnitude of these changes without the need to dive on to the supplementary materials.	Revised: "Moreover, while annual water temperature increased (approx. +0.18 °C per decade, totaling +5.7°C over 32 years) and annual river discharge decreased (approx. -4% per decade, totaling -13% over 32 years) (Figure 3, Table S2), these hydroclimatic trends did not show a direct, strong correlation with the timing or magnitude of these decadal metabolic shifts (Figure S8), suggesting that their direct influence on the observed changes in annual NEP magnitude was secondary to the effects of re-oligotrophication and community shifts."
L417. Therefore…oligotrophication implies an improvement of water quality but a decrease in the capacity of the river to act as a CO2 sink. This seems like an important take home message.	Revised: An important implication of these findings is that while re-oligotrophication signifies an improvement in certain aspects of water quality (e.g., reduced nutrient loads and algal biomass), it can concurrently lead to a decreased capacity of the river to act as a CO2 sink and may alter the balance of CO2 sources towards greater internal contributions when the river is a source.
L422. Which "linkage" and "the variation" of what? Do you mean, that a decrease in FCO2 over time cannot be attributed to changes in groundwater inputs because discharge showed no clear decreases over time?	"This linkage" refers to the idea that reduced discharge leads to reduced lateral CO2 transport. The sentence means that while reduced discharge seasonally might correlate with lower external CO2 (Fig S7 shows Q vs External), the inter-annual trend of decreasing discharge doesn't strongly explain the inter-annual trend of decreasing external CO2 (Fig S8 shows weak R2 for annual Q vs annual External). This was already revised for General Comment 1.
L423-425. According to figure S7 and S8 discharge explains from 19-26% of external CO2 sources.	Revised: "...annual discharge explains approximately 19% (R2 = 0.19, p = 0.013) of the inter-annual variation in external CO2 source magnitude, while annual temperature shows a weaker, non-significant relationship (R2 = 0.06, p = 0.196) (Figure S8)."
L439. "low frequency variation" of what?	Revised: "Trend analysis of groundwater table levels in France over the past 30 years shows low-frequency variations, specifically multi-annual (~7 years) and decadal (~17 years) cycles in groundwater storage (Baulon et al., 2022)."
L439-450. Overall, I found this part of the discussion quite speculative. Afterall, why groundwater CO2 fluxes have decreased so in the last decades? How the observed multi-annual low frequency variations relate with the results presented? How can these groundwater inputs be constrained in future studies?	As detailed in our response to your General Comment (and further supported by our new analysis of local groundwater pH and alkalinity trends presented in New Figure S_groundwater), our revised discussion now more robustly addresses the potential reasons for a decrease in groundwater CO 2 fluxes.
L462-464. Not sure what the authors mean in this sentence. Could you rewrite?	Revised: "While our study infers changes in external CO2 sources, a full understanding of carbon transfer at the groundwater-river interface requires more extensive DIC data from groundwaters and riparian zones (Deirmendjian &

	Abril, 2018; Duvert et al., 2018). Future work should focus on obtaining such data to better quantify these fluxes."
L455-468. Could you be more specific about what do you mean with "new exploration" and "extrapolation on river networks"?	Revised: "We also suggest that further analyses of similar long-term, high-frequency datasets from other large rivers, where available are crucial. Moreover, developing approaches to extrapolate findings from such detailed site studies across diverse river networks, integrating them with spatial hydrological and ecological data, will be key to better understanding and predicting how global changes will influence the balance between internal and external CO_2 production at broader scales, ultimately refining estimates of the role of rivers in the global carbon budget."
Figure S7. Mention the color ramp in the caption.	Revised Figure S7 Caption: "Relationship of daily fluxes (FCO_2, External CO_2, Minus_NEP) with daily discharge (left column) and daily water temperature (right column). Points are colored by year, as indicated by the color bar. Red lines are linear regressions with R^2 and p-values shown."
Figure S9. What means NGF? Check units for annual external CO_2 (g C m^{-2} y^{-1})	NGF stands for "Nivellement Général de la France," which is the official vertical datum for France. Revised Figure S9 Caption: "Multi-annual patterns of annual external CO_2 source in the Loire River (this study) and mean annual groundwater level in France (data extracted from Baulon et al., (2022)). Groundwater level is expressed in meters relative to the Nivellement Général de la France (m NGF) datum.

Reviewer 2

General comments

Nguyen et al. report on long-term CO_2 and metabolism data in a large temperate river. They show long-term shifts in the autotrophic/heterotrophic balance of the river, which they link to management changes that occurred since the early 2000s (i.e. lower nutrient inputs leading to lower GPP and a more heterotrophic river). Their data also suggest strong seasonal variations in both metabolism and CO_2 emissions. Interestingly, while the river becomes more heterotrophic over time, hence with increased internal CO_2 production, CO_2 emissions tend to decrease in parallel. The authors attribute this decline in emissions to a decrease in external CO_2 inputs at the catchment scale. They also show extreme year-to-year variability in both river metabolism and CO_2 emissions, a finding that confirms the limitations of single-year studies and the need for long-term data where feasible.

This is a potentially great study based on a rare long-term dataset of paired O_2 and (indirect) CO_2 measurements. The study offers insights into the links between river metabolism and CO_2 emissions, an emerging research area that is receiving some attention in smaller streams but remains unexplored in larger rivers – even less so over such extended timeframes. The findings should be of broad interest to the community, as improving our understanding of the temporal variations (diel, seasonal and interannual) in the source/sink status of rivers is a priority. However, several aspects of the paper require some improvement before it can be published.

Response: We thank Reviewer 2 for constructive feedback and recognition of the study's potential. We have addressed the general and specific comments below.

First, the Methods section lacks details that can help readers assess the robustness of the datasets and the validity of the methods. I see some details are in the SI, but some information should appear in the main text. Details regarding any QAQC of the pH, alkalinity and oxygen datasets are crucial, as the entire study relies on these parameters. Importantly, the authors mention somewhere that older membrane sensors were replaced with optical sensors in 2008. This date coincides with a clear step increase in pH values and a resulting decrease in pCO_2 values (as expected) and CO_2 emissions (Figure 2), which brings up a crucial question: how much of the observed long-term decrease in CO_2 emissions is a result of this sensor change? How confident are the authors about the continuity of the pH measurements across the entire time-series?

Response: While EDF performed regular calibrations (mentioned in SI S2), subtle inter-sensor changes in precision/accuracy post-2008 are hard to rule out completely as contributors to the observed 2008 shift. We have conducted a detailed comparison with independent grab sample data Water Agency (monthly data, www.naiades.eaufrance.fr) to assess the continuity of measurements across this transition (New Figure S_grab_sampling).

We found a small shift in pH residuals from -0.17 (±0.35) pH units before 2008 to -0.10 (±0.32) pH units after 2008. Post-2008 optical sensor indicates less differences with the grab samples, but both continuous sensor data from EDF and grab samples data from water agency are well consistent for the long term trend. Therefore the sensor change may have contributed to the step observed in 2008, but it is unlikely to be the sole driver of the entire multi-decadal pCO_2 trend.

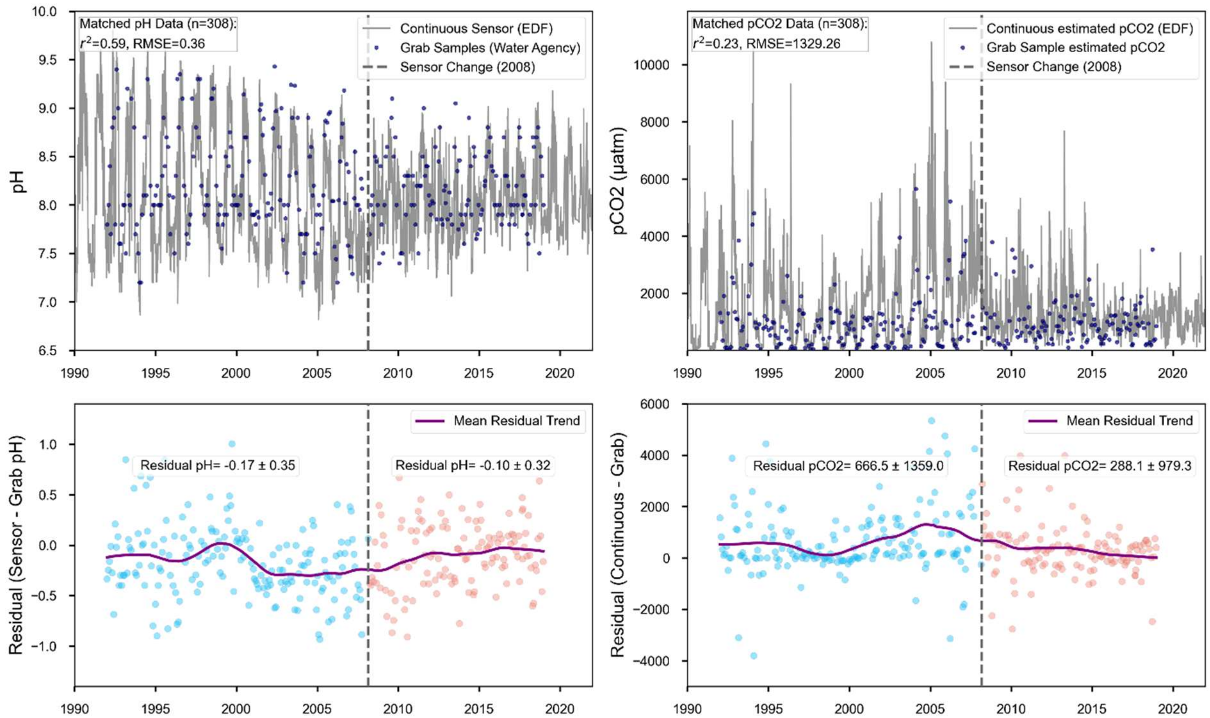


Figure S_grab_sampling: Comparison of continuous sensor (EDF) vs. grab sample (water agency) for pH and estimated pCO2 in 1990-2021. Bottom panels show residuals (Sensor - Grab) with points colored pre/post sensor change in 2008, a LOWESS trend, and per-period mean residual ± SD.

On another note, the metabolism modelling using streamMetabolizer is described too briefly, and there are no details on the conversion of metabolism fluxes (expressed in O2 units) into CO2 fluxes (unless I have missed it). The indirect estimation of k600 is another area that needs to be further scrutinised – where and when do the two methods (empirical models versus metabolism) yield the largest discrepancies, and why might that be?

Response: The reference Diamond et al. (2021) contains the detailed model structure, priors, and fitting used for the Loire. We can briefly reiterate key aspects.

"Briefly, the Bayesian model used a state-space formulation to estimate daily GPP, ER, and K600 from from hourly DO. Priors K600 was constrained as a function of daily discharge and depth using empirical relationships (Raymond et al., 2012) to improve identifiability, as detailed in Diamond et al. (2021). Model convergence and fit were assessed using standard Bayesian diagnostic tools (e.g., R-hat statistics, visual inspection of observed vs. modeled DO)."

Regarding O2 to C conversion: "GPP and ER are estimated by streamMetabolizer in oxygen units (g O2 m-2 d-1) and subsequently converted to carbon units (g C m-2 d-1) for calculating NEP and for comparison with FCO2. In this study, we assumed a 1:1 molar ratio for O2:C for both GPP (photosynthetic quotient, PQ = 1) and ER (respiratory quotient, RQ = 1), and applied uniformly across all years and trophic regimes.

Regarding k600: Supplementary Figure S5 compares streamMetabolizer K600 (converted to k600 by multiplying by depth) with the mean and range of seven Raymond et al. (2012) equations. The Raymond et al. (2012) k600 estimates tended to be higher in summer and lower in winter compared to those estimated by the StreamMetabolizer model. Reasons for discrepancies:

- Raymond equations are empirical, based on hydraulic variables (Q, depth, slope, velocity). They do not consider the DO data like streamMetabolizer
- In summer (low flow, lower turbulence), Raymond eqs might overestimate if turbulence is low. streamMetabolizer K600 might be lower if diel DO is strong and suggests less reaeration is needed to explain DO patterns.
- In winter (high flow, high turbulence), Raymond might give high k600. If metabolism is very low, streamMetabolizer K600 might also be high to balance any residual DO variation, or it could be poorly constrained. We will add a brief synthesis of this to the main Methods.

Revised: "The streamMetabolizer-derived K600 values, while generally within the same order of magnitude as those from empirical hydraulic equations (Supplementary Figure S5), showed some seasonal deviations. Specifically, k600 values from empirical equations tended to be higher in summer and lower in winter compared to streamMetabolizer estimates (Supplementary Information, Section S3). Such discrepancies can arise because streamMetabolizer co-estimates K600 with GPP and ER based on fitting observed DO dynamics, making its K600 sensitive to the strength of the biological signal, whereas empirical equations rely solely on hydraulic proxies for turbulence. For instance, during low-flow summer periods with strong metabolic signals, streamMetabolizer might yield a different K600 than hydraulic equations if the latter do not fully capture all drivers of gas exchange (e.g., wind effects, thermal stratification). We chose the streamMetabolizer K600 to maintain internal consistency between the metabolic and FCO2 calculations."

Second, some of the interpretations, particularly regarding the potential drivers of the observed long-term decrease in CO2 emissions, need to be expanded. The authors mention a decrease in external CO2 sources, but could this trend instead reflect carbonate buffering processes, i.e. the conversion of some of the CO2 into alkalinity? As the authors do not seem to have collected any pCO2 data in shallow groundwater, could the above hypothesis be tested by updating their mass balance in Figure 5 with an additional term representing the downstream export of CO2 and/or DIC? Alternatively, have the authors considered any concomitant land use change that could explain some of the decline in CO2 inputs? Or could the decline be, at least in part, an artefact related to the sensor change in 2008?

Response:

About carbonate buffering, our data (Figure 2a) show that alkalinity in the Loire at the study site has been relatively stable over the 32 years, with no significant long-term increasing trend that would support a major shift towards CO2 sequestration as HCO3-.

About adding downstream export of CO2/DIC in mass balance (Figure 5) would make it a full DIC budget for the river reach, which is a different scope. We do not have downstream DIC concentration data at the same temporal resolution to robustly calculate DIC export over 32 years. So we prefer to keep Figure 5 as it is.

On another note, I would invite the authors to discuss how representative their single measurement site is of the whole river system. Are the findings scalable to the entire river system? Some of the authors have worked on spatial variations in metabolism across river networks, so this should be a relatively easy addition.

Response: Our site is in the middle Loire, the freshwater zone. Dynamics upstream (smaller, steeper tributaries) and further downstream (closer to estuary, larger, slower) will differ. However, the re-oligotrophication and macrophyte shift were observed over large sections of this part of the river (Minaudo 2015). So, the temporal trends driven by these broad-scale changes are likely representative for similar large, temperate river sections that underwent such changes. However, the absolute magnitudes of FCO2, NEP, and their balance will vary spatially. The mechanisms and patterns (e.g., importance of trophlux state transitions, hysteresis, impact of trophic shifts on CO2 sources) are likely relevant and scalable concepts for understanding other large river sections.

Specific comments from reviewer 2	**Responses from Nguyen et al.,**
Abstract L30. Remove "was" in "the degree of which was depended"	Revised: "the degree of which depended"
Introduction L37-38. This statement should be updated with the more recent estimates in Liu et al. (2022). https://www.pnas.org/doi/abs/10.1073/pnas.2106322119	Revised: "Rivers are significant conduits for carbon from terrestrial landscapes to the ocean and atmosphere, with global CO_2 evasion from rivers estimated at approximately 2.0 ± 0.2 Pg C yr^{-1} (Liu et al., 2022), representing a substantial component of inland water carbon fluxes."
L47-49. While I agree that seasonal and interannual variations remain under-studied, this statement omits recent studies that report paired CO_2 and DO measurements for several years. Perhaps tone this statement down a bit.	Revised: "Consequently, while progress has been made (e.g., Young et al., 2025, on a four-year study), a comprehensive understanding of the full spectrum of temporal variability (seasonal to multi-decadal) in FCO_2 and its sources remains limited, particularly for large river systems. This hinders accurate assessment of their role under changing climatic and environmental conditions..."
L77. "most eutrophic river"	"one of the most eutrophic rivers in Europe "
L86. "NEP decreased by approximately 10%"	"an approximate 10% decrease in NEP"
Methods L117-119. What sensors were used for pH and DO measurements? What was their measurement range, accuracy, frequency of cleaning and calibration? Some of this information is in the SI but much of it should be moved to the main text.	We agree that some sensor information is important for assessing data quality, but believe that extensive technical specifications would detract from the main focus of this ecological study. We propose adding the following concise information to the main text while maintaining detailed specifications in the supplementary materials. Please check previous responses for detail answer.

L121-122. What is the uncertainty of indirectly estimating alkalinity on pCO2 estimates?	"The daily TA data in this study were estimated from daily EC with an average error of 190 μmol L-1, leading to an uncertainty in pCO2 estimation. PyCO2SYS can estimate pCO2 uncertainty by propagating the TA uncertainty (Humphreys et al., 2022). Uncertainty of estimated TA leads to ±11% uncertainty in pCO2 estimation..."
L131-132. "To avoid unrealistic estimates of K600, values were constrained…"	Done
L130-141. This section on metabolic modelling needs additional methodological details. Please specify the priors used in the model, how model performance was assessed, etc.	We will add brief details on priors and model assessment, referencing Diamond et al. (2021) for full specifics
L153. Remove "the" before FCO2.	Done
L156. Remove one of the occurrences of "with"	Done: "multiplying river depth with K600".
L157-159. It would help to show a scatter plot comparing the k600 values from the Raymond models with those from streamMetabolizer, so readers can see how well they match. Also, please explain why the two estimates might be different at high and low flow. As importantly, please clarify which quotients were used to convert O2 fluxes to CO2 fluxes, and explain how those values were chosen.	We add a scatter plot for k600 below (S_k600_compare) Why estimates differ at high/low flow: Addressed in General Comment We will add a summary to Methods. O2 to CO2 quotients: Addressed in General Comment

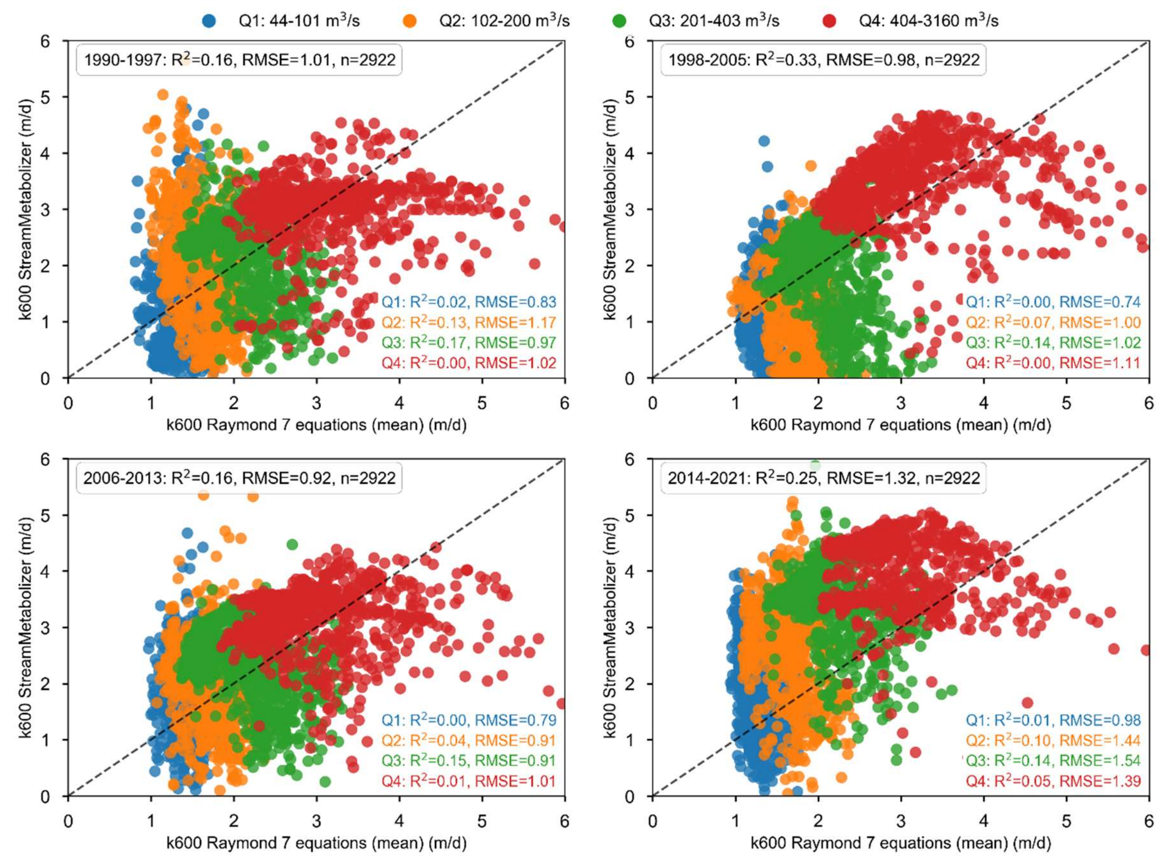
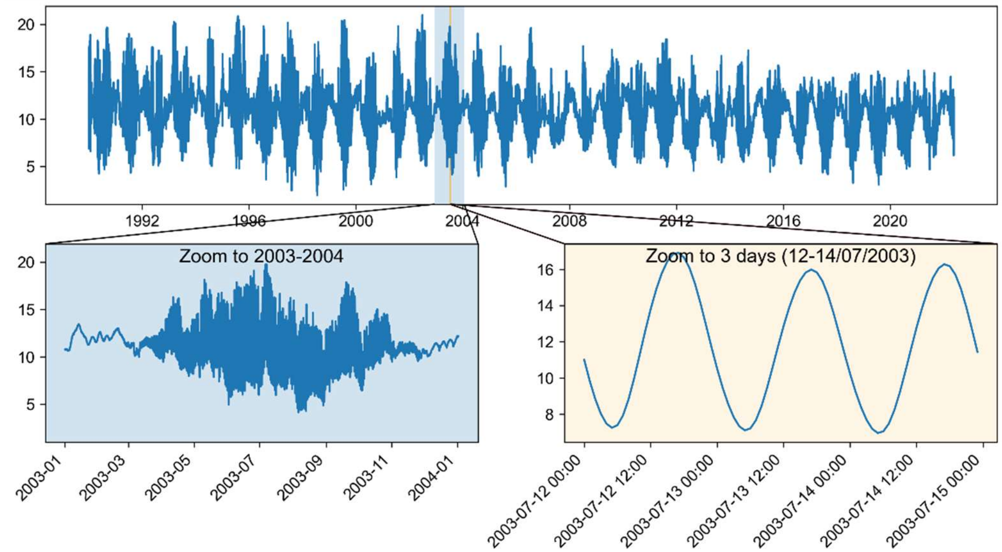


Figure S_k600_compare: Compare k600 between mean of 7 equations Raymond 2012 and StreamMetabolizer for 1990-2021. Colors indicate discharge quantiles (Q1-Q4, legend above). R2 and RMSE shown per discharge quantile range.

Results

L206. It would be useful to show (at least in the SI) a plot of the long-term DO time-series.

We add a figure to the SI showing the long-term (32-year) time series of hourly DO concentrations.

	 Figure S_DO: Hourly DO (mgO_2/L) in Dampierre station in 1990-2021
L210. "autotrophic/heterotrophic for NEP, sink/source for FCO_2"	Done
L236. "while there was"	Done
L229. Figure 2. It seems that most of the decrease in pCO_2 is driven by an increase in pH rather than a change in alkalinity. How confident are the authors that this is not related to the sensor replacement that occurred in 2008?	We will propose text revisions for Methods and Discussion to address this explicitly Addressed in General Comment
Discussion L336-337. What explanations can the authors put forward to explain such decreases in external CO_2 sources? Are they discharge-related? Do they relate to biogenic or geogenic sources?	These questions are at the core of the discussion on external CO_2 sources. Discharge-related: Just partly, but inter-annual Q trend only explains ~19% of external CO_2 trend Biogenic/geogenic sources: External CO_2 comes from soil respiration (biogenic), groundwater (can be biogenic from overlying soils or geogenic from carbonate/silicate weathering reactions

Can the decline be the result of carbonate buffering, i.e. some of the CO_2 is converted to alkalinity follwoing increases in pH?	that also produce CO_2 or consume it and affect DIC). A decrease could mean less soil CO_2 production/transport, or changes in groundwater chemistry/pathways. Carbonate buffering: Addressed in General Comment. Stable alkalinity suggests this is not an accelerating in-river sink mechanism explaining the FCO_2 trend. The Discussion section (Lines 418-450) explores these, focusing on groundwater levels and potential land-use changes.
L374-377. Solano et al. (2023) present a figure summarising the range of percent contributions of NEP to FCO_2 across the literature, which could provide useful context here. https://aslopubs.onlinelibrary.wiley.com/doi/full/10.1002/lno.12334	Revised: "These values fall within the broad range reported for various river systems. For example, Solano et al. (2023), in their synthesis of literature, show the contribution of net ecosystem respiration to CO_2 emissions varies extensively, from very low percentages (e.g., <10%) in some systems to values exceeding 100% in others where internal metabolism dominates evasion, with many temperate rivers exhibiting contributions in the 20% to 70% range depending on river size, trophic state, and hydrological conditions. Our long-term data for a single large river highlight that substantial temporal variation in this ratio can occur even within one system due to both seasonal dynamics and multi-decadal environmental changes."
L418. OK, some of my earlier questions are addressed in this section. I still think further discussion is needed, particularly regarding the potential influence of the sensor change in 2008, and the role of carbonate buffering. On this second point, I suggest integrating downstream export into the budgets presented in Figure 5.	Adding downstream DIC/CO_2 export would change it to a full reach-scale DIC budget. This is a larger scope and requires downstream concentration data we do not have for a 32-year consistent budget. The focus of Figure 5 is to visually explain the components leading to the observed FCO_2 (or uptake).
L440. Add "scales" after multi-annual and decadal.	Revised: "...multi-annual (~7 years) and decadal (~17 years) scales (Baulon et al., 2022)."