nature portfolio

Peer Review File

Andean headwater and piedmont streams are hot spots of carbon dioxide and methane emissions in the Amazon basin

Web links to the author's journal account have been redacted from the decision letters as indicated to maintain confidentiality

14th Sep 22

Dear Dr Borges,

Please accept my apologies for the delay in obtaining reports for your manuscript titled "Andean headwater and piedmont streams are hot spots of CO2 and CH4 emissions within the Amazon basin". Your manuscript has now been seen by 3 reviewers, and I include their comments at the end of this message. They find your work of interest, but some important points are raised. We are interested in the possibility of publishing your study in Communications Earth & Environment, but would like to consider your responses to these concerns and assess a revised manuscript before we make a final decision on publication.

We therefore invite you to revise and resubmit your manuscript, along with a point-by-point response that takes into account the points raised. In particular, we require that you address the concerns regarding the upscaling method, which may mean demonstrating that the current approach is robust, applying a new approach or removing this aspect of the analysis from the manuscript. We also require that you quantify and communicate the limitations and uncertainties in your analysis. In addition, we ask that you provide an in-depth and balanced discussion of the related literature and clearly communicate the advance that your findings offer beyond this earlier work. Please highlight all changes in the manuscript text file.

We are committed to providing a fair and constructive peer-review process. Please don't hesitate to contact us if you wish to discuss the revision in more detail.

Please use the following link to submit your revised manuscript, point-by-point response to the referees' comments (which should be in a separate document to any cover letter) and the completed checklist:
[link redacted]
** This url links to your confidential home page and associated information about manuscripts you may have submitted or be reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage first **

We hope to receive your revised paper within six weeks; please let us know if you aren't able to submit it within this time so that we can discuss how best to proceed. If we don't hear from you, and the revision process takes significantly longer, we may close your file. In this event, we will still be happy to reconsider your paper at a later date, as long as nothing similar has been accepted for publication at Communications Earth & Environment or published elsewhere in the meantime.

We understand that due to the current global situation, the time required for revision may be longer than usual. We would appreciate it if you could keep us informed about an estimated timescale for resubmission, to facilitate our planning. Of course, if you are unable to estimate, we are happy to accommodate necessary extensions nevertheless.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further. We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Best regards,

Clare

Clare Davis, PhD
Senior Editor
Communications Earth & Environment

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REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

“Andean headwater and piedmont streams are hot spots of CO_2 and CH_4 emissions within the Amazon basin” is a well-written manuscript that brings meaningful contributions to the current knowledge regarding GHG emissions in the Amazon Basin. In this paper, Chiriboga and Borges use estimated k values and GHG concentration measurements to calculate fluxes in headwater and piedmont streams, and compare it to emissions in lowland rivers. Fluxes per m^2 appear to be very significant and suggest that regional emissions in the Andes may account to 35 to 72% of the Amazon basin scale integrated fluvial emissions for CO_2 and CH_4, respectively. For comparison, the estimated CH_4 fluxes (~0.4 Tg CH_4 per year) are equivalent to one third of global CH_4 emissions estimated by Bastviken et al. 2011 (although more recent estimations suggest that global rivers and streams may emit around 7 to 25 Tg of CH_4 per year, e.g. Stanley et al. 2016).
I believe this manuscript will be adequate for publication after the authors have included a more comprehensive discussion regarding the limitations and uncertainties associated to the methods employed in this study. In general, estimations made in this study are highly dependent on k values estimated from RiverATLAS. Still, the limitations and setbacks of using estimated k values are not discussed within the text. I would expect a section in this article delving into that. Also, it intrigues me why the authors have chosen to not include some floating chamber measurements in the experimental design, as it would be great if at least

some site-specific k were compared to the estimated k to improve computation of the estimation errors. Considering the k-values you got, I guess flow velocities and turbulence was the main reason for not using the floating chambers, but I imagine it would still be possible to use it in some sampling stations.

While measured data shows high standard deviations, it seems that the application of estimated k values has masked some of the intrinsic variation that you usually see in measurements using site-specific calculated k values. Confidence intervals in almost all upscaling attempts are broader than the ones presented in this study. Also, limitations of the upscaling method are not clearly presented. Is the vegetation cover in the area really allowing us to have good estimates for the entire Amazon region? From Figure S8 and Figure S3 it seems that only 2 sites are in non-forested areas. What are the sources for the equation and associated errors in line 437? What are the limitation and biases in applying these estimates? Considering all the generalizations being made here, I think it would be good leave this clear to the readers.

In summary, including a section clearly depicting the biases and limitations of the estimates shown here would greatly improve the manuscript.

Please find below some specific considerations:

Lines 40-41: Some of the CO_2 emissions estimates (such as ref. 5 or Sawakuchi et al. 2017) suggest that the upper boundary could be even higher. I guess 1.8 came from the mean of the ranges presented on ref. 5 (or maybe from Raymond et al. 2013). Is there a reason for not considering the upper limit at ~2.5 PgCy-1 ?

Lines 43-44: I agree that this comparison is meaningful, as wetland emissions account for roughly 40% of CH_4 aquatic emissions, but this may not be the case for a reader that is not familiar with this information. This is just a suggestion, but maybe there is a better way of « anchoring » the significance of these emissions.

Lines 44-48: There is a bit of a circular reasoning here, as you attribute a « downward revision » to explain the « downward revision ».

Lines 71-72: When you mention enhances in this sentence, it gives me a sense of «higher emissions » in these areas compared to mountainous rivers. Since I am not a native english speaker,I may be the one to blame here, but is there a better choice of words to avoid any confusion?

Lines 79-83: Raymond et al. 2013 got the 0.5 PgC yr-1 rate from Richey et al. 2002. So you should probably consider values provided by Sawakuchi et al. 2017, which revised Jeffrey Richey's estimates. Also, I believe it is a better practice to cite the original source, and not (only) the paper that compiled the results.

Lines 83-84: Melack et al. 2004 is still highly cited, but I guess Pangala et al. 2017 (Nature) and Basso et al. 2021 (Communication Earth and Environment) have brought important contributions to these estimates. So why not consider these updated values ?

Lines 116-122: Maybe it is worth rephrasing this part a bit, as it feels like you are explaining the variability in the obtained data with estimated values that derive from them. Also, it would be good if you could calculate how much of the estimated FCH_4 and FCO_2 depends on k values (a sensitivity test). Here is a case where site-specific k measurements using floating chambers would strengthen you conclusions a lot.

Lines 123 - It's « terra firme »

Line 230 and others – I think it is better if you always specify if CH_4 refers to concentrations, production, fluxes, etc. (e.g. The higher CH_4 in piedmont rivers)

Line 361 – Just a detail that is included in the reference material, but I think it is worth

mentioning how you created the headspace here (I guess it was by injecting 30ml of nitrogen, right?)
Line 417 to 421 – Would it be possible to perform a sensitivity test to measure the effect of this approximation?
Georeferenced dataset - Would it be possible to also include slope and elevation parameters extracted from RIVERAtlas in this data-set? I guess it would improve reproducibility and data-usage.

Reviewer #2 (Remarks to the Author):

The manuscript entitled "Andean headwater and piedmont streams are hot spots of CO_2 and CH_4 emissions within the Amazon basin" brings important data for the GHG emissions in the headwaters of Amazon basin. The manuscript is well written and clear; however, I have a major concern about the upscaling method. The authors had upscaled the emissions for CH_4, CO_2 and N_2O for the Amazon basin using slope as an integrative method, but the relationship between the CH_4 and N_2O and slope looks terrible, as shown in the figures 4 and 5. Therefore, I feel that using slope for upscaling the emissions could lead for a huge misconception of the real emissions. Therefore, the authors should consider to remove the upscaling data or using another method for upscaling the emissions, since the concentrations and flux data are important to be published.

Line 124: It is important to mention that at high elevation, there are lower inputs of organic matter when compared to lowland regions. In addition, I think that there is lower organic carbon in soils, which should be confirmed through other studies.

Line 155: I don't agree that catchment slope could be used as an integrative metric for processes that affect GHGs, once the availability of carbon may not change across the slope, considering anthropogenic land-uses and anthropogenic impacts.

Line 241: Stream CO_2 are generally produced in the groundwater. See Johnson et al 2008 https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2008GL034619

Line 264: As stated before, the slope could be used as an integrative metric for extrapolation if the land-use are similar across the sites, especially because of the carbon inputs. If this is the case, then yes, you could use slope as an integrative metric, but this should be clear in the manuscript, including the limitations.

Andean headwater and piedmont streams are hot spots of CO_2 and CH_4 emissions within the Amazon basin

Chiriboga and Borges

Manuscript #: COMMSENV-22-0527-T

Summary

Chiriboga and Borges present an exciting dataset of GHG (CO_2, CH_4, and N_2O) from Andean headwater streams in the Amazon basin. The authors state these are the first measurements in these streams, which begs for a literature review or demonstration to support this claim, particularly as they cite and use data from Richey et al. later in the analysis. The authors evaluate spatial patterns of GHG concentration, finding catchment slope and elevation as important drivers of gas concentrations and as proxy for ecosystem and watershed processing of C and N. Finally the authors calculate emissions, using modeled estimates of gas exchange velocity, k, and evaluate the fluxes from these under-measured streams compared to streams in the Amazon as a whole. The paper is a clear presentation of data, application of emission calculation, and upscaling with important context around inland water emissions, and particularly from the tropics.

These data are timely, as there is rapidly growing interest in GHG data and improving spatial and temporal resolution of estimates from inland waters and will be beneficial to the community. The data are a clear demonstration of existing data collection and analysis from streams and rivers, but there are interesting conclusions and linkages to spatial and watershed scale processes elucidated through remote sensing.

General comments

I find one major criticism related to the science of the paper and most comments (detailed below) pertain to writing style and voice used in the paper. Throughout the introduction and results/discussion, the paper explores possible drivers and mechanistic processes regulating the gas data measured in the streams. As is the case with gases that have biogenic origins, making conclusions or inferences about processes governing gas concentrations are readily made. However, as these rates, processes, and/or fluxes were not empirically measured, I recommend the authors to focus more on what the GHG data itself indicates, and less on ecosystem and watershed scale processes that were not measured.

This leads to my criticism about voice. Throughout the paper, conditional language is used (e.g., ‘could’, ‘should’, ‘might’, ‘likely’). Shifting a larger focus towards what the gas data by itself indicates will allow the authors to edit or remove these conditional phrases. One analysis or interpretation of gas data that could enhance the evaluation of the data that was collected is presented in Vachon et al. (2020), where the paired measurements of O_2 and CO_2 inform in-stream and watershed scale metabolic and hydrologic processes. One particular confounding aspect of the analysis relates to O_2 measurements and the time of day O_2 was measured (also specified below). O_2 can vary strongly in streams, particularly in productive streams with low k, and in these streams, acknowledgement of this variation should be included in the discussion.

Last, the figures present various statistical regressions that are not detailed in the methods and are presented in the text as correlated or related processes. For the sake of transparency and rigor, some evaluation and presentation of these statistical fits should be introduced and justified, including model comparisons. The relationships appear to be general trends with little to be interpreted from what the regression means to say from the data. In this case, they are intuitive fits, but not necessarily meaningful.

Tables, Figures, and Supplements

Figure 3: Unclear what ‘property-property’ in the caption specifically refers to. For panel b, would log-transforming the axes help visualize the clustering of points at lower concentrations? Is there any presentation or evaluation of the regression lines shown in this and other figures? Or model comparison between linear, logarithmic, or polynomial fits to a given relationship?

Figure 4: I really like showing the median for each stream type overlain on each panel. However, the medians are difficult to see in certain panels (b, c, e). Could these points be layered on top of the raw data? Or could the transparency of the raw data be increased? Some visualization technique to highlight the median values amidst the raw data. See comment for Fig 3 about regression fits.

Figure 5: See above comment about units for k600. My personal preference is for m/d and the literature seems to have coalesced around these units as well. Perhaps an editorial note, but in the caption, the first panel that should be mentioned is a), whereas here, panel d is mentioned first. Please re-arrange the caption or figures to alphabetical order.

Figure S3: what is the spatial resolution of the land cover data? Are these showing the upstream area of each of the sampling points?

Figure S4: Similar to my main criticism, this paper is showing correlation but not a causal linkage between the patterns shown here. I suggest changing ‘affect’ to ‘are correlated’ in the caption to represent the lack of a mechanistic linkage in the data of this paper. These panels are also subject to the use of regression lines without discussion of their relevance.

Figure S7: ‘natural vegetation’ seems an odd classification. Is this all forested or paramo?

Figure S8: In the caption, state what ‘nd’ means.

Figure S9: similar to Figure 4, the points for the median are difficult to see.

Line specific comments

L27, L29: ‘areal fluxes’ rather than per m^2

L32: ‘In contrast…’

L39: inland waters <u>do</u> contribute a large quantity of GHG to the atmosphere, but the contributions of these bodies are not responsible for or contributing to climate change. Rather, this is a flux with large uncertainty and likely to change in a warmer and more variable climate. This introduction suggests that rivers and streams are contributing to climate change, but I think the value of this research is in quantifying uncertainty of GHGs from a new part of the globe and particularly the tropics.
L51: what does total (44%) refer to? Total land area in the tropics?
L54: a citation at the end of this sentence would be helpful. There is a growing body of work that suggests in-stream metabolism and net C mineralization is temperature independent, despite thermodynamic predictions (Marzolf and Ardón 2021; Bernhardt et al. 2022) (albeit many of the data arguing this are from outside the tropics)
L59: end the topic sentence at 'reasons' and start a new sentence with 'One'.
L62: remove 'could'. Authors cite papers in this sentence which demonstrate their statement and no need for the conditional qualifier
L67 (and elsewhere): reviewer preference against starting sentences with 'this', 'these', 'there', etc. Help your reader and be specific about what you are referring to.
L82: may depend on journal, but 'Neotropics' is often capitalized

L105: how was the values of atmospheric GHG chosen?
L111: 'The patterns of percent oxygen saturation (%O2)…'. Do the authors consider time of day into the analysis? O2 can vary strongly during the day due to photosynthesis and respiration, and time of sampling may influence the patterns found here.
L116: 'inversely correlated', delete 'individually'. 'These patterns…'
L118: In this analysis, I think the authors should highlight that k is contributing to most of the fluxes. A variety of input and output fluxes are mentioned but not empirically measured, whereas k is known and the variation in k is well documented. I argue more discussion or more weight should be added to the known attribution of k to FGHGs.
L125, L137: are fringing wetlands similar to floodplain wetlands?
L140: change 'nil' to 'zero'.
L149: change 'levels' to 'concentrations'
L152: Stating k varies with gradient and slope seems like a circular argument, as those terms are part of the equation to estimate k. Not sure the purpose of the second sentence in this paragraph, if the topic of the paragraph is k.
L173: Part of the variation in DIN might be similar to processes in Central America and the tropical N paradox (Brookshire et al. 2012a, b), particularly where fertilizer inputs are negligible.
L198: '…Inmiyana, and Tiputini…'
L243: what is the 'important depth'?
L250: possible hypothesis: lowlands have a greater fraction of wetlands and anoxic environments (e.g. river sediments, water-logged soils) where denitrification, as well as incomplete denitrification, occurs and provides a pool of N2O to rivers. This also corroborates with higher pCO2 in lowland streams with higher %N2O (reduction of NO3 to N2O produces CO2) and

lower CH4, as NO3 is being reduced in these environments rather than the redox ladder is using the least efficient electron transfer (ie methanogenesis).
L269: is there evidence for ebullitive CH4, either in the literature or in personal observations? Particularly from low velocity areas?
L273: Units are confusing and interacting. Can k be presented in m/d? Upscaling daily k to annual emissions is an easier transition than cm/h.
L314 (and earlier in introduction): first-time seems strong. Particularly when data from other studies is used (e.g. Richey et al.) to compare and inform gaps in this analysis

L338: '…catchment ranges from 100 to 6300 masl…'
L346: while not part of the Amazonian drainage, there are studies from the Ecuadorian paramo that are worth including as part of the discussion, particularly in regards to variability in k and pCO2 (Schneider et al. 2020; Whitmore et al. 2021). In particular, Schneider et al. discuss transport vs. source limitation of GHG emissions. Incorporating discussion of where k or [GHG] does and does not limit FGHG would be a valuable contribution, particularly given the spatial variation across sites and the importance of elevation and slope of FGHG.
L364: how were duplicate samples evaluated? What was the CV among duplicates and what was the post hoc evaluation of high variation between duplicates, if necessary?
L392: are these additional samples or the same samples taken for gases?
L437: It is not stated, but is k here converted to k_{gas} from k_{600}? If so, that should be stated and the equations to convert between Sc numbers should be shown.

References

Bernhardt ES, Savoy P, Vlah MJ, et al (2022) Light and flow regimes regulate the metabolism of rivers. Proc Natl Acad Sci U S A 119:e2121976119. https://doi.org/10.1073/pnas.2121976119

Brookshire EN, Gerber S, Menge DN, Hedin LO (2012a) Large losses of inorganic nitrogen from tropical rainforests suggest a lack of nitrogen limitation. Ecol Lett 15:9–16. https://doi.org/10.1111/j.1461-0248.2011.01701.x

Brookshire ENJ, Hedin LO, Newbold JD, et al (2012b) Sustained losses of bioavailable nitrogen from montane tropical forests. Nat Geosci 5:123–126. https://doi.org/10.1038/ngeo1372

Marzolf NS, Ardón M (2021) Ecosystem metabolism in tropical streams and rivers: a review and synthesis. Limnol Oceanogr 66:1627–1638. https://doi.org/10.1002/lno.11707

Schneider CL, Herrera M, Raisle ML, et al (2020) Carbon Dioxide (CO 2) Fluxes From Terrestrial and Aquatic Environments in a High-Altitude Tropical Catchment. J Geophys Res Biogeosciences 125:e2020JG005844. https://doi.org/10.1029/2020JG005844

Vachon D, Sadro S, Bogard MJ, et al (2020) Paired O 2 –CO 2 measurements provide emergent insights into aquatic ecosystem function. Limnol Oceanogr Lett 5:287–294. https://doi.org/10.1002/lol2.10135

Whitmore KM, Stewart N, Encalada AC, et al (2021) Spatiotemporal variability of gas transfer velocity in a tropical high-elevation stream using two independent methods. Ecosphere 12:e03647. https://doi.org/10.1002/ecs2.3647

Reviewer #1

“Andean headwater and piedmont streams are hot spots of CO2 and CH4 emissions within the Amazon basin” is a well-written manuscript that brings meaningful contributions to the current knowledge regarding GHG emissions in the Amazon Basin. In this paper, Chiriboga and Borges use estimated k values and GHG concentration measurements to calculate fluxes in headwater and piedmont streams, and compare it to emissions in lowland rivers. Fluxes per m^2 appear to be very significant and suggest that regional emissions in the Andes may account to 35 to 72% of the Amazon basin scale integrated fluvial emissions for CO2 and CH4, respectively. For comparison, the estimated CH4 fluxes (~0.4 Tg CH4 per year) are equivalent to one third of global CH4 emissions estimated by Bastviken et al. 2011 (although more recent estimations suggest that global rivers and streams may emit around 7 to 25 Tg of CH4 per year, e.g. Stanley et al. 2016).

Reply: We thank the reviewer for taking the time to evaluate our work.

I believe this manuscript will be adequate for publication after the authors have included a more comprehensive discussion regarding the limitations and uncertainties associated to the methods employed in this study. In general, estimations made in this study are highly dependent on k values estimated from RiverATLAS. Still, the limitations and setbacks of using estimated k values are not discussed within the text. I would expect a section in this article delving into that. Also, it intrigues me why the authors have chosen to not include some floating chamber measurements in the experimental design, as it would be great if at least some site-specific k were compared to the estimated k to improve computation of the estimation errors. Considering the k-values you got, I guess flow velocities and turbulence was the main reason for not using the floating chambers, but I imagine it would still be possible to use it in some sampling stations.

Reply: We did not measure the fluxes with floating chambers, so these data are not available. But, we followed the reviewer’s recommendation and we added a detailed section (**L394-500**) of 1,270 words which discusses the limitations of the methods to derive gas transfer velocities. We compiled studies that compare the floating chamber measurements (as suggested by the review) to k computed with the parameterization of Raymond et al. (2012) (the method we used in the paper) and there is a general agreement. This information was added **L479**.

While measured data shows high standard deviations, it seems that the application of estimated k values has masked some of the intrinsic variation that you usually see in measurements using site-specific calculated k values. Confidence intervals in almost all upscaling attempts are broader than the ones presented in this study. Also, limitations of the upscaling method are not clearly presented. Is the vegetation cover in the area really allowing us to have good estimates for the entire Amazon region?

From Figure S8 and Figure S3 it seems that only 2 sites are in non-forested areas. What are the sources for the equation and associated errors in line 437? What are the limitation and biases in applying these estimates? Considering all the generalizations being made here, I think it would be good leave this clear to the readers.

Reply: We did not estimate the fluxes to the entire Amazon based on the data collected in Ecuadorian streams, we additionally compiled all available data from literature (totaling 403 data points for pCO_2 and 472 data points for CH_4, refer to Fig. 4). We have added a detailed section (**L394-500**) of 1,270 words that discusses all of the uncertainties related to the scaling exercise. We discuss limitations of the method to scale the gas concentrations, the k and the stream surface area.

In summary, including a section clearly depicting the biases and limitations of the estimates shown here would greatly improve the manuscript.

Reply: We followed the reviewer's suggestion and we have added a detailed section (**L394-500**) of 1,270 words that discusses all of the uncertainties related to the scaling exercise.

Please find below some specific considerations:

Lines 40-41: Some of the CO2 emissions estimates (such as ref. 5 or Sawakuchi et al. 2017) suggest that the upper boundary could be even higher. I guess 1.8 came from the mean of the ranges presented on ref. 5 (or maybe from Raymond et al. 2013). Is there a reason for not considering the upper limit at ~2.5 PgCy-1 ?

Reply: We have included the higher bound from the paper of Sawakuchi (**L40**).

Lines 43-44: I agree that this comparison is meaningful, as wetland emissions account for roughly 40% of CH4 aquatic emissions, but this may not be the case for a reader that is not familiar with this information. This is just a suggestion, but maybe there is a better way of « anchoring » the significance of these emissions.

Reply: We added a sentence to convey this information (**L44**).

Lines 44-48: There is a bit of a circular reasoning here, as you attribute a « downward revision » to explain the « downward revision ».

Reply: We state that the total emission was revised downward because the one of the terms of the computation was revised downward. This seems to us to be a linear causal relation and not a circular reasoning.

Lines 71-72: When you mention enhances in this sentence, it gives me a sense of «higher emissions » in these areas compared to mountainous rivers. Since I am not a native english speaker,I may be the one to blame here, but is there a better choice of words to avoid any confusion?

Reply: We use the wording "enhance" because we want to state that the presence of wetlands leads to high CO_2 and CH_4 concentrations in rivers and this in turn leads to high CO_2 and CH_4 emissions. Lowland rivers in Ecuador were indeed characterized by higher CO_2 and CH_4 fluxes, please refer to Figure S9 (of the revised version).

Lines 79-83: Raymond et al. 2013 got the 0.5 PgC yr-1 rate from Richey et al. 2002. So you should probably consider values provided by Sawakuchi et al. 2017, which revised Jeffrey Richey's estimates. Also, I believe it is a better practice to cite the original source, and not (only) the paper that compiled the results.

Reply: As suggested by the reviewer, we refer to the original paper, and we added the reference to Richey et al. (2002) and we included the citation to the Swakuchi 2017 paper (**L40**).

Lines 83-84: Melack et al. 2004 is still highly cited, but I guess Pangala et al. 2017 (Nature) and Basso et al. 2021 (Communication Earth and Environment) have brought important contributions to these estimates. So why not consider these updated values ?

Reply: We added these two references (**L97)**.

Lines 116-122: Maybe it is worth rephrasing this part a bit, as it feels like you are explaining the variability in the obtained data with estimated values that derive from them. Also, it would be good if you could calculate how much of the estimated FCH4 and FCO2 depends on k values (a sensitivity test). Here is a case where site-specific k measurements using floating chambers would strengthen you conclusions a lot.

Reply: We did not measure the fluxes with floating chambers. But, we followed the reviewers recommendation and we added a detailed section (**L257-293**) of 466 words and with a new Figure (Fig. S10) which discusses the drivers of the emissions in terms of transport-limitation and supply-limitation.

Lines 123 - It's « terra firme »

Reply: Changed as requested (**L141**).

Line 230 and others – I think it is better if you always specify if CH4 refers to concentrations, production, fluxes, etc. (e.g. The higher CH4 in piedmont rivers)

Reply: We have carefully checked the whole text to specify whether we refer to concentrations or fluxes.

Line 361 – Just a detail that is included in the reference material, but I think it is worth mentioning how you created the headspace here (I guess it was by injecting 30ml of nitrogen, right?)

Reply: We have added the information on volume of headspace (**L567**).

Line 417 to 421 – Would it be possible to perform a sensitivity test to measure the effect of this approximation?

Reply: We addressed this point in three ways. We have made an uncertainty analysis by propagating errors (**L661**); we have added a detailed discussion on the relevance of using slope as a predictor (**L400-460** with 749 words); we have added an analysis of predicting with a MLR with other terms that shows this did not improve substantially the predictions with the linear regression based on slope (**L458**).

Georeferenced dataset - Would it be possible to also include slope and elevation parameters extracted from RIVERAtlas in this data-set? I guess it would improve reproducibility and data-usage.

Reply: We have added the sub-set of RiverATLAS data corresponding to our sampling sites.

Reviewer #2

The manuscript entitled "Andean headwater and piedmont streams are hot spots of CO2 and CH4 emissions within the Amazon basin" brings important data for the GHG emissions in the headwaters of Amazon basin. The manuscript is well written and clear; however, I have a major concern about the upscaling method. The authors had upscaled the emissions for CH4, CO2 and N2O for the Amazon basin using slope as an integrative method, but the relationship between the CH4 and N2O and slope looks terrible, as shown in the figures 4 and 5. Therefore, I feel that using slope for upscaling the emissions could lead for a huge misconception of the real emissions. Therefore, the authors should consider to remove the upscaling data or using another method for upscaling the emissions, since the concentrations and flux data are important to be published.

Reply: We thank the reviewer for taking the time to evaluate our work.

Line 124: It is important to mention that at high elevation, there are lower inputs of organic matter when compared to lowland regions. In addition, I think that there is lower organic carbon in soils, which should be confirmed through other studies.

Reply: We agree with the reviewer and we have added a detailed section (**L400-460** with 749 words) that discussed how slope (and elevation) modulate carbon cycling in streams, including soil organic carbon content.

Line 155: I don't agree that catchment slope could be used as an integrative metric for processes that affect GHGs, once the availability of carbon may not change across the slope, considering anthropogenic land-uses and anthropogenic impacts.

Reply: We added a section (**L400-460** with 749 words) justifying the use of catchment slope as an integrative metric. Please note that previous multi-parametric models of pCO_2 at large scale (continent or global) showed that catchment slope explains to most variance and is consequently the most important predictor (Lauerwald et al. 2013; 2015; Liu et al. 2022).

Line 241: Stream CO2 are generally produced in the groundwater. See Johnson et al 2008 https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2008GL034619

Reply: The Jonhson paper is used in the discussion (Ref 28)

Line 264: As stated before, the slope could be used as an integrative metric for extrapolation if the land-use are similar across the sites, especially because of the carbon inputs. If this is the case, then yes, you could use slope as an integrative metric, but this should be clear in the manuscript, including the limitations.

Reply: We added a section (**L400-460** with 749 words) that addresses the reviewer's concerns and justifies the use of catchment slope as an integrative metric.

Reviewer #3

Summary

Chiriboga and Borges present an exciting dataset of GHG (CO2, CH4, and N2O) from Andean headwater streams in the Amazon basin. The authors state these are the first measurements in these streams, which begs for a literature review or demonstration to support this claim, particularly as they cite and use data from Richey et al. later in the analysis. The authors evaluate spatial patterns of GHG concentration, finding catchment slope and elevation as important drivers of gas concentrations and as proxy for ecosystem and watershed processing of C and N. Finally the authors calculate emissions, using modeled estimates of gas exchange velocity, k, and evaluate the fluxes from these under-measured streams compared to streams in the Amazon as a whole. The paper is a clear presentation of data, application of emission calculation, and upscaling with important context around inland water emissions, and particularly from the tropics.

Reply: We have removed from the present version of the manuscript prior statements on "first measurements". We are obviously not the first to measure CO_2, CH_4 and N_2O in the Amazonian rivers and streams, this would have been a totally ridiculous claim, and we certainly did not intend to mislead readers into believing this. We stated these were the "first measurements" in the Andean mountainous streams.

These data are timely, as there is rapidly growing interest in GHG data and improving spatial and temporal resolution of estimates from inland waters and will be beneficial to the community. The data are a clear demonstration of existing data collection and analysis from streams and rivers, but there are interesting conclusions and linkages to spatial and watershed scale processes elucidated through remote sensing.

Reply: We thank the reviewer for taking the time to evaluate our work.

General comments

I find one major criticism related to the science of the paper and most comments (detailed below) pertain to writing style and voice used in the paper. Throughout the introduction and results/discussion, the paper explores possible drivers and mechanistic processes regulating the gas data measured in the streams. As is the case with gases that have biogenic origins, making conclusions or inferences about processes governing gas concentrations are readily made.

However, as these rates, processes, and/or fluxes were not empirically measured, I recommend the authors to focus more on what the GHG data itself indicates, and less on ecosystem and watershed scale processes that were not measured.

Reply: For a journal such as *COMMSENV*, a manuscript cannot be limited to a simple data description, and an in-depth and novel interpretation of the data is required. We developed a detailed discussion of the more surprising, unexpected and intriguing aspects of the data-set. For instance, the persistent and generalized under-saturation in N_2O in streams came as a surprise, and called for an explanation related to nitrogen cycling in the soils of the watershed. Similarly, 3 of the streams were on the contrary characterized by comparatively very strong N_2O over-saturation, and this seemed related to palm oil plantations, requiring a few words of explanation. Overall, we find our discussion balanced and a useful contribution for the community. Admittedly, we did not measure all of the processes cited in the discussion, but, this is not a reason for not including this information in the discussion.

This leads to my criticism about voice. Throughout the paper, conditional language is used (e.g., 'could', 'should', 'might', 'likely'). Shifting a larger focus towards what the gas data by itself indicates will allow the authors to edit or remove these conditional phrases. One analysis or interpretation of gas data that could enhance the evaluation of the data that was collected is presented in Vachon et al. (2020), where the paired measurements of O2 and CO2 inform in-stream and watershed scale metabolic and hydrologic processes. One particular confounding aspect of the analysis relates to O2 measurements and the time of day O2 was measured (also specified below). O2 can vary strongly in streams, particularly in productive streams with low k, and in these streams, acknowledgement of this variation should be included in the discussion.

Reply: Wording and tone of a manuscript is rather subjective and should be left to the responsibility of the authors, although we thank the reviewer for the suggestion. Since we make several hypotheses to try to explain the more noteworthy and unexpected features in the data set, we used a conditional tone that seemed more adequate than an assertive tone.

We thank the reviewer for pointing out the paper of Vachon et al. (2020), however, we prefer to keep our paper focused on GHG dynamics and not add other topics such as stream metabolism. Note that the proposed approach is best suited for paired O_2-CO_2 from high-resolution continuous time series, for example refer to Marzolf et al (2022). Our "snap-shot" discontinuous measurements (designed to describe broad altitudinal gradients and inter-stream variability) are not ideal for paired O_2-CO_2 analysis as envisaged, for instance, by Marzolf et al (2022).

Last, the figures present various statistical regressions that are not detailed in the methods and are presented in the text as correlated or related processes. For the sake of transparency and rigor, some evaluation and presentation of these statistical fits should be introduced and justified, including model comparisons. The relationships appear to be general trends with little to be interpreted from what the regression means to say from the data. In this case, they are intuitive fits, but not necessarily meaningful.

Reply: The reviewer is absolutely right. In the present version of the ms, we have included the statistics of the fits in Table S4.

Tables, Figures, and Supplements

Figure 3: Unclear what ‘property-property’ in the caption specifically refers to. For panel b, would log-transforming the axes help visualize the clustering of points at lower concentrations?

Reply: We have removed the wording “property-property” (**L1016**).

Is there any presentation or evaluation of the regression lines shown in this and other figures? Or model comparison between linear, logarithmic, or polynomial fits to a given relationship?

Reply: We have included the statistics of the fits in Table S4.

Figure 4: I really like showing the median for each stream type overlain on each panel. However, the medians are difficult to see in certain panels (b, c, e). Could these points be layered on top of the raw data? Or could the transparency of the raw data be increased? Some visualization technique to highlight the median values amidst the raw data. See comment for Fig 3 about regression fits.

Reply: We tried the suggestions of the reviewer and other options and the rendering did not improve compared to our original version, that was kept unchanged.

We have included the statistics of the fits in Table S4.

Figure 5: See above comment about units for k600. My personal preference is for m/d and the literature seems to have coalesced around these units as well.

Reply: We have changed the units of k_{600} from cm/h to m/d.

Perhaps an editorial note, but in the caption, the first panel that should be mentioned is a), whereas here, panel d is mentioned first.

Please re-arrange the caption or figures to alphabetical order.

Reply: We changed the caption accordingly.

Figure S3: what is the spatial resolution of the land cover data? Are these showing the upstream area of each of the sampling points?

Reply: The land cover data are derived from RiverATLAS. We have clarified the text that the cover is upstream of the sampled stream.

Figure S4: Similar to my main criticism, this paper is showing correlation but not a causal linkage between the patterns shown here. I suggest changing ‘affect’ to ‘are correlated’ in the caption to represent the lack of a mechanistic linkage in the data of this paper. These panels are also subject to the use of regression lines without discussion of their relevance.

Reply: We agree with the reviewer that correlation does not imply causation. We change wording to “correlated”.

We have included the statistics of the fits in Table S4.

Figure S7: ‘natural vegetation’ seems an odd classification. Is this all forested or paramo?

Reply: We used “natural” by opposition to human impacted land-use such as plam oil plantation. We changed the caption to clarify this.

Figure S8: In the caption, state what ‘nd’ means.

Reply: Changed as requested.

Figure S9: similar to Figure 4, the points for the median are difficult to see.

Reply: We tried the suggestions of the reviewer and other options and the rendering did not improve compared to our original version, that was left unchanged.

Line specific comments

L27, L29: ‘areal fluxes’ rather than per m2

Reply: Changed as requested (**L26,28**).

L32: ‘In contrast…’

Reply: Changed as requested (**L32**).

L39: inland waters do contribute a large quantity of GHG to the atmosphere, but the contributions of these bodies are not responsible for or contributing to climate change. Rather, this is a flux with large uncertainty and likely to change in a warmer and more variable climate.

This introduction suggests that rivers and streams are contributing to climate change, but I think the value of this research is in quantifying uncertainty of GHGs from a new part of the globe and particularly the tropics.

Reply: We do not mention “climate change” in the introduction but we (partly) agree with the reviewer that inland water GHGs emissions do not contribute to the increase of the atmospheric GHG content. But this is not the entirely complete picture since N2O emissions from streams are in part driven by N inputs from fertilizers. The emissions of CO2 and CH4 from reservoirs (included in inland waters) are “human-made”. There is growing evidence that eutrophicated rivers and lakes/ponds emit substantially more CH4 (also “human-made”). There is evidence that eutrophicated rivers and lakes/ponds absorb CO_2 (so by definition a sink of anthropogenic CO2, albeit probably marginal). So there is some space for nuance, and this is an emerging research topic, although not necessarily in the scope of the introduction of this manuscript. We have changed the text to mention that GHG emissions from inland waters affect the natural and anthropogenic components (**L38)**.

L51: what does total (44%) refer to? Total land area in the tropics?

Reply: We clarified text (**L54**).

L54: a citation at the end of this sentence would be helpful. There is a growing body of work that suggests in-stream metabolism and net C mineralization is temperature independent, despite thermodynamic predictions (Marzolf and Ardón 2021; Bernhardt et al. 2022) (albeit many of the data arguing this are from outside the tropics)

Reply: We agree with the reviewer that for streams in the tropical climate zone, differences in metabolism are not mainly driven by temperature variations. But, metabolism of tropical inland water differs from streams in other climate zones (temperate and boreal) in part due to climate (light and temperature).

L59: end the topic sentence at 'reasons' and start a new sentence with 'One'.

Reply: Changed as requested, **L62**.

L62: remove 'could'. Authors cite papers in this sentence which demonstrate their statement and no need for the conditional qualifier

Reply: Changed as requested, **L65**.

L67 (and elsewhere): reviewer preference against starting sentences with 'this', 'these', 'there', etc. Help your reader and be specific about what you are referring to.

Reply: Changed as requested, here (**L70**) and elswhere.

L82: may depend on journal, but 'Neotropics' is often capitalized

Reply: Changed as requested, **L87**.

L105: how was the values of atmospheric GHG chosen?

Reply: We added this information **L117**.

L111: 'The patterns of percent oxygen saturation (%O2)…'. Do the authors consider time of day into the analysis? O2 can vary strongly during the day due to photosynthesis and respiration, and time of sampling may influence the patterns found here.

Reply: We did not consider time of day in the analysis of O2 and CO2. But we now mention this possible caveats **L550**.

L116: 'inversely correlated', delete 'individually'. 'These patterns…'

Reply: Changed as requested, **L248**.

L118: In this analysis, I think the authors should highlight that k is contributing to most of the fluxes. A variety of input and output fluxes are mentioned but not empirically measured, whereas k is known and the variation in k is well documented. I argue more discussion or more weight should be added to the known attribution of k to FGHGs.

Reply: We added a detailed section (**L257-293**) of 466 words and with a new Figure (Fig. S10) which discusses the drivers of the emissions in terms of transport-limitation and supply-limitation.

L125, L137: are fringing wetlands similar to floodplain wetlands?

Reply: fringing wetlands also include less conspicuous riparian macrophytes. The term comes from the cited reference.

L140: change 'nil' to 'zero'.

Reply: Changed as requested, **L157**.

L149: change 'levels' to 'concentrations'

Reply: Changed as requested, **L168**.

L152: Stating k varies with gradient and slope seems like a circular argument, as those terms are part of the equation to estimate k. Not sure the purpose of the second sentence in this paragraph, if the topic of the paragraph is k.

Reply: We do not agree with the reviewer. *k* is computed from stream slope (at reach level). Upstream terrain slope is not a variable in the computation of *k*. So both terms are independent.

L173: Part of the variation in DIN might be similar to processes in Central America and the tropical N paradox (Brookshire et al. 2012a, b), particularly where fertilizer inputs are negligible.

Reply: The reviewer is absolutely right and we are grateful for pointing out the work of Brookshire et al. (2012a, b) that is cited in the present version of the manuscript **L193**.

L198: '…Inmiyana, and Tiputini…'

Reply: Changed as requested, **L207**.

L243: what is the 'important depth'?

Reply: Changed by "deep water column", **L320**.

L250: possible hypothesis: lowlands have a greater fraction of wetlands and anoxic environments (e.g. river sediments, water-logged soils) where denitrification, as well as incomplete denitrification, occurs and provides a pool of N_2O to rivers. This also corroborates with higher pCO2 in lowland streams with higher %N2O (reduction of NO3 to N2O produces CO2) and lower CH4, as NO3 is being reduced in these environments rather than the redox ladder is using the least efficient electron transfer (ie methanogenesis).

Reply: This is an interesting possible hypothesis. However, denitrification also occurs in highlands (since it is the most plausible process removing N_2O and creating N_2O under-saturation), consequently, denitrification is not confined to lowlands. So, we prefer to leave this an open question, although the altitudinal pattern of N_2O in streams followed the altitudinal pattern of N_2O soil fluxes, which we find comforting.

L269: is there evidence for ebullitive CH4, either in the literature or in personal observations?

Particularly from low velocity areas?

Reply: We did not measure CH_4 ebullition. In the Amazon, ebullitive CH_4 fluxes were reported in literature for floodplains (summarized by Melack et al. 2004), and seldom in streams and rivers. Data from recent papers (Barbosa, Sawakushi) does not allow to conclude on the relative importance of ebullition compared to diffusion of CH_4 in streams.

L273: Units are confusing and interacting. Can k be presented in m/d? Upscaling daily k to annual emissions is an easier transition than cm/h.

Reply: We have changed the units of k_{600} from cm/h to m/d.

L314 (and earlier in introduction): first-time seems strong. Particularly when data from other studies is used (e.g. Richey et al.) to compare and inform gaps in this analysis.

Reply: We have removed from the present version of the manuscript prior statements on “first measurements” (here and elsewhere in text). We are obviously not the first to measure CO_2, CH_4 and N_2O in the Amazonian rivers and streams, and we certainly did not intend to mislead readers into believing this. This would have been a totally ridiculous claim, especially given there is a whole section of the manuscript devoted to the comparison of data-sets from previous studies that were obviously referenced (section “Comparison of stream GHG content with Central Amazon basin”). We included in the present version of the manuscript reference to the work of Schneider et al. that we were sincerely unaware of at the time of submitting our manuscript. Since the Editor asked us to clearly communicate the advance that our findings offer beyond earlier work, we stated that we provide the first large scale survey of CO_2, CH_4 and N_2O exchange between Andean mountainous streams of the Amazon River basin and the atmosphere (**L504**). Richey *et al.* (1988,2002) reported data in Central Amazon, downstream of San Antonio do Iça (-68°E) located more than 1,000 km (downstream) from the Andes Cordillera.

L338: ‘…catchment ranges from 100 to 6300 masl…’

Reply: Changed as suggested, **L528**.

L346: while not part of the Amazonian drainage, there are studies from the Ecuadorian paramo that are worth including as part of the discussion, particularly in regards to variability in k and pCO2 (Schneider et al. 2020; Whitmore et al. 2021). In particular, Schneider et al. discuss transport vs. source limitation of GHG emissions. Incorporating discussion of where k or [GHG] does and does not limit FGHG would be a valuable contribution, particularly given the spatial variation across sites and the importance of elevation and slope of FGHG.

Reply: We included in the present version of the manuscript reference to the work of Schneider et al. and Whitmore et al. that we were sincerely unaware of at the time of submitting our manuscript.

We added a detailed section (**L257-293**) of 466 words and with a new Figure (Fig. S10) which discusses the drivers of the emissions in terms of transport-limitation and supply-limitation.

L364: how were duplicate samples evaluated? What was the CV among duplicates and what was the post hoc evaluation of high variation between duplicates, if necessary?

Reply: We averaged the duplicate samples. No post hoc evaluation was necessary, both values always converged. The CVs are reported **L573**.

L392: are these additional samples or the same samples taken for gases?

Reply: We sampled water separately in a 2L polyethylene bottle. We added this information to text **L595**.

L437: It is not stated, but is k here converted to kgas from k600? If so, that should be stated and the equations to convert between Sc numbers should be shown.

Reply: k was of course converted from k_{600}; we added this information as requested **L654**.

References

Marzolf, N.S. *et al.* Partitioning inorganic carbon fluxes from paired O_2-CO_2 gas measurements in a Neotropical headwater stream, Costa Rica. *Biogeochemistry* **160**, 259–273 (2022).

15th Feb 23

Dear Dr Borges,

Your manuscript titled "Andean headwater and piedmont streams are hot spots of CO2 and CH4 emissions within the Amazon basin" has now been seen by our reviewers, whose comments appear below. In light of their advice I am delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Earth & Environment under the open access CC BY license (Creative Commons Attribution v4.0 International License).

We therefore invite you to revise your paper one last time to address the remaining concerns of our reviewers. In particular, we require that you clearly communicate the limitations of using catchment slope as an integrative metric, and check that data are consistent between equations, tables and figures. In addition, we ask that you provide comprehensive details of how errors and uncertainties in your analysis were calculated. At the same time we ask that you edit your manuscript to comply with our format requirements and to maximise the accessibility and therefore the impact of your work.

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Best regards,

Clare Davis, PhD
Senior Editor
Communications Earth & Environment

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REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

As I've mentioned in my first review, this is a well-written manuscript that brings meaningful contributions to the current knowledge regarding GHG emissions in the Amazon Basin.

The authors have addressed most of my concerns regarding the previous version, with substantial improvements in reproducibility the discussion regarding uncertainties associated to the methods employed in this study. However, I have additional considerations regarding this revised version:

The author's justification of using catchment slope as an integrative metric may not hold strong enough evidence, as the relationship between slope and fluxes in their data is not strong. Consider, for example the huge variability around the regression lines (e.g., r-squared of 0.21 and 0.42 for CH_4). To avoid misleading readers, the limitations of this approach should be clearly stated in the abstract and introduction. This is also valid for the figure titles (e.g., strong elevation gradients).

I also got a bit lost on where the equations from the section “Computation and upscaling of FCO_2, FCH_4, FN_2O” came from. The authors mention Figure 4, but the statistical data provided in Table S4 is a bit different from those equations. Also, how were errors calculated here? Given the huge variability around the regression line, the model seems to be missing a lot of uncertainty. But still, I am not a statistician and may be missing something.

The word correlation is being used loosely in some cases (e.g. line 248 of the marked up version). M. M. Mukaka (2012, Malawi Medical Journal; 24(3)) provides a very “easy to digest” guide on how to properly use the term “correlation” in research and I suggest following those good practices.

Also, the "fit" plotted in Figure 4b may not be represented in Table S6, as the equation y = -0.6519*x + 3.656 describes a linear fit, which may not match the data in Table S6. It appears that there is a similar issue in Figure 4a.

Lastly, there is one "(" missing in the first line of Table S2.

Reviewer #2 (Remarks to the Author):

The manuscript entitled "Andean headwater and piedmont streams are hot spots of CO2 and CH4 emissions within the Amazon basin"have greatly improved since the last version. My main concern about the upscaling techniques was addressed by adding an entire section in the discussion, explaining the uncertainties about the technique. I don't have any further comments and congratulate the authors for this amazing work.

Reviewer #3 (Remarks to the Author):

Andean headwater and piedmont streams are hot spots of CO2 and CH4 emissions within the Amazon basin

COMMSENV-22-0527A

Chiriboga and Borges

The paper provides important GHG concentrations, emissions, and drivers from an understudied region in the tropics. The authors report these data with discussion of uncertainty, particularly in gas exchange, as well as catchment-scale drivers that relate to GHG variability at multiple scales.

The authors have made substantial changes from the three reviews and I appreciate their efforts, particularly given the nit-picky nature of my initial review, for which I could have been less so. This is true for figure aesthetics, which can be a lot of work, and providing statistical tables.

I appreciate the comments back re: conditional language if that is the author’s decision. I address one case where conditional language could be removed in the line specific comments, but not meant as a required change, simply an example.

I recommend adding 1-2 sentences of broader context to the beginning of the abstract. Could be as simple as copy and pasting the first sentence in the introduction to the abstract, and including some background on why the Andean headwaters are important to study.

Below are minor line specific comments addressing typos or adding points of clarification.
L16: 'increased exponentially with decreasing elevation' is potentially confusing. Maybe 'concentrations were exponentially higher at lower elevation'?
L38: delete 'could'?
L42: what does significant mean here? Why are 10x lower emissions significant? I agree CH4 emissions from rivers are important but I'm not sure what the word significant means in this context.
L48: not sure including the previous estimate is useful. Just report the downward revised estimate and the citation
L51: delete 'extremely'
L51: Suggest a new paragraph when talking about the tropics. This is a change in topic in the paragraph from global to a region of the globe. A topic sentence that introduces latitudinal differences in emissions would help make the transition (e.g. Raymond et al. 2013).
L64: you could pose differences across longitudinal gradients as another source of uncertainty, as was done for within and among river basins.
L86: this paragraph on the Amazon could be moved to the methods (~L527). Maybe the highpoints can be included into the introduction to exemplify the large contributions of the tropics to GHG emissions from rivers.
L106: can the authors expand the end of this paragraph. They are certainly doing more in the paper, and they can introduce some of the comparisons and scaling they do here.
L124: 'increased' and 'decreased', see comment from L16
L141: personal preference for an Oxford comma after 'wetlands'
L147: Returning to the original submission, the critique of conditional language, here and elsewhere. For example, this can be re-written: "Rates of respiration and methanogenesis in soils and riverine sediments are positively related to temperature suggesting warmer conditions in the lowland rivers contributed to higher pCO2 and dissolved CH4 concentration values. Indeed, pCO2 and dissolved CH4 concentrations increased exponentially with water temperature (Fig. 2d,h), indicating water temperature explains the decreasing pattern of pCO2 and dissolved CH4 concentration with elevation".
L152: what does 'marked' mean? Measurable? Statistically significant?
L281: Is there an extra 0 in the elevation?
L328: maybe 'hypothesis' rather than 'explanation'? Also, wouldn't lower k in lowland streams/rivers (on average) lead to greater %N2O?
L418: Seems this sentence refutes including the discussion of the models immediately above it. It might be better to lead with the idea that predicting [GHG] is complex and has high uncertainty, then include some of the variables that others have used in their models to demonstrate the complexity, possible redundancy, and challenges of predicting at global scales. This will also allow you to highlight your approach and why it is different, and perhaps better. Further, the paragraph is also over a page long, I suggest breaking into 2 or more paragraphs.

Reviewer #1 (Reply)

As I've mentioned in my first review, this is a well-written manuscript that brings meaningful contributions to the current knowledge regarding GHG emissions in the Amazon Basin.

Reply: We warmly thank the reviewer for taking the time to evaluate our submission a second time, and providing additional comments to improve the content of the manuscript.

The authors have addressed most of my concerns regarding the previous version, with substantial improvements in reproducibility the discussion regarding uncertainties associated to the methods employed in this study. However, I have additional considerations regarding this revised version:

The author's justification of using catchment slope as an integrative metric may not hold strong enough evidence, as the relationship between slope and fluxes in their data is not strong. Consider, for example the huge variability around the regression lines (e.g., r-squared of 0.21 and 0.42 for CH4). To avoid misleading readers, the limitations of this approach should be clearly stated in the abstract and introduction. This is also valid for the figure titles (e.g., strong elevation gradients).

Reply: We agree with the reviewer that the relations of CH_4 and other variables show scatter. Indeed, CH_4 in rivers and streams shows strong small scale variations both in time and space. This does not mean that our choice of model is incorrect but that the model does not allow to capture all of the variability in the data. Yet, we provided theoretical arguments that justify the choice of this model. We also tested other models and there was no improvement. The statistical validity of a linear regression is given by the p-value at a given significance level and not by the r-squared alone (that needs to be checked against a Table of critical values of regression coefficients). All of the linear regressions used in our model were significant at 0.05 level. For both regressions of CH_4 versus slope p<0.0001. For equation (2), a linear regression is statistically significant for a r^2>0.013 (critical value) for a sample of n=305 and the computed r^2 is 0.42. For equation (3), a linear regression is statistically significant for a r^2>0.022 (critical value) for a sample of n=174 and the computed r^2 is 0.21. It was not our intention to mislead readers, on the contrary, we have very transparently presented all of the equations (with related statistics) used to scale the fluxes. We do find appropriate to mention the limitation of the approach in the abstract that is supposed to convey the main findings (and for which there is a strict limit of the number of words). We do not find appropriate to mention the limitation of the approach in the “Introduction” before we present the results and the method to scale the data. We did include in the manuscirpt during the previous round of review a lengthy discussion on the pro and cons of using slope to scale the GHG fluxes.

I also got a bit lost on where the equations from the section "Computation and upscaling of FCO2, FCH4, FN2O" came from. The authors mention Figure 4, but the statistical data provided in Table S4 is a bit different from those equations. Also, how were errors calculated here? Given the huge variability around the regression line, the model seems to be missing a lot of uncertainty. But still, I am not a statistician and may be missing something.

Reply: The regressions given in Supplemental Table 4 correspond to the fits shown in figures. There is a difference with the equations given in material & methods because the figures give the slope in degrees while in HydroATLAS the slope is reported as degrees x 10. For consistency, we used the data in HydroATLAS with the original units and gave the equations in the M&M in those units. This is mentioned in text that reads below equation (1) "where CS is the catchment slope (x10°)"

The errors on the coefficients of the regression (Y-intercept and slope angle) were computed by least-square linear regression, in a traditional way with a standard software (Graphpad Prism). Please note that the regressions were computed on the log transformed data.

The word correlation is being used loosely in some cases (e.g. line 248 of the marked up version). M. M. Mukaka (2012, Malawi Medical Journal; 24(3)) provides a very "easy to digest" guide on how to properly use the term "correlation" in research and I suggest following those good practices.

Reply: We thank the reviewer for pointing out the reference of Mukaka (2012). However, we were puzzled by the "rule of thumb for interpreting the size of a correlation coefficient" given in Table 1 of the cited reference, because the statistical significance of r-square depends on the size of the sample and is determined by the comparison with a critical value that varies with sample size (tabulated in manuals of statistics). For instance, a r^2 of 0.9 is not significant at 0.05 level for n=4; but r^2 of 0.2 is significant (albeit low) for n=25. This is why the use of the p-value is extremely useful (as reported in Supplemental Table 4). In text, we used wording "correlation" or "correlated" appropriately given the p-values reported in Supplemental Table 4.

Also, the "fit" plotted in Figure 4b may not be represented in Table S6, as the equation y = -0.6519*x + 3.656 describes a linear fit, which may not match the data in Table S6. It appears that there is a similar issue in Figure 4a.

Reply: The linear regression was computed to the log transformed data (for both X and Y), while the curve in the figure is represented with the data in linear scale, so it becomes non-linear.

Lastly, there is one "(" missing in the first line of Table S2.

Reply: This was corrected as suggested.

Reviewer #2 (Reply)

The manuscript entitled "Andean headwater and piedmont streams are hot spots of CO2 and CH4 emissions within the Amazon basin"have greatly improved since the last version. My main concern about the upscaling techniques was addressed by adding an entire section in the discussion, explaining the uncertainties about the technique. I don't have any further comments and congratulate the authors for this amazing work.

Reply: We warmly thank the reviewer for taking the time to evaluate our submission a second time, and providing a positive recommendation.

Reviewer #3 (Reply)

The paper provides important GHG concentrations, emissions, and drivers from an understudied region in the tropics. The authors report these data with discussion of uncertainty, particularly in gas exchange, as well as catchment-scale drivers that relate to GHG variability at multiple scales.

The authors have made substantial changes from the three reviews and I appreciate their efforts, particularly given the nit-picky nature of my initial review, for which I could have been less so. This is true for figure aesthetics, which can be a lot of work, and providing statistical tables.

I appreciate the comments back re: conditional language if that is the author's decision. I address one case where conditional language could be removed in the line specific comments, but not meant as a required change, simply an example.

Reply: We warmly thank the reviewer for taking the time to evaluate our submission a second time, and providing additional comments to improve the content of the manuscript.

I recommend adding 1-2 sentences of broader context to the beginning of the abstract. Could be as simple as copy and pasting the first sentence in the introduction to the abstract, and including some background on why the Andean headwaters are important to study.

Reply: The abstract was modified accordingly.

Below are minor line specific comments addressing typos or adding points of clarification.

L16: 'increased exponentially with decreasing elevation' is potentially confusing. Maybe 'concentrations were exponentially higher at lower elevation'?

Reply: The text was modified accordingly.

L38: delete 'could'?

Reply: The text was modified accordingly.

L42: what does significant mean here? Why are 10x lower emissions significant? I agree CH4 emissions from rivers are important but I'm not sure what the word significant means in this context.

Reply: Word significant was removed.

L48: not sure including the previous estimate is useful. Just report the downward revised estimate and the citation

Reply: We kept both numbers because removing the previous estimate as suggested would have made awkward the following sentence.

L51: delete ‘extremely’

Reply: The text was modified accordingly.

L51: Suggest a new paragraph when talking about the tropics. This is a change in topic in the paragraph from global to a region of the globe. A topic sentence that introduces latitudinal differences in emissions would help make the transition (e.g. Raymond et al. 2013).

Reply: A paragraph start was added. We did not add a sentence since the latitudinal differences are the topic itself of the paragraph.

L64: you could pose differences across longitudinal gradients as another source of uncertainty, as was done for within and among river basins.

Reply: The reviewer is absolutely right; we modified the sentence accordingly.

L86: this paragraph on the Amazon could be moved to the methods (~L527). Maybe the highpoints can be included into the introduction to exemplify the large contributions of the tropics to GHG emissions from rivers.

Reply: We did not find a satisfactory way to split the information so as to move part of it to the methods, so we preferred to keep the original text.

L106: can the authors expand the end of this paragraph. They are certainly doing more in the paper, and they can introduce some of the comparisons and scaling they do here.

Reply: The reviewer is absolutely right; we added a sentence to provide a more complete account of the content of manuscript.

L124: ‘increased’ and ‘decreased’, see comment from L16

Reply: The text was modified accordingly.

L141: personal preference for an Oxford comma after ‘wetlands’

Reply: The text was modified accordingly.

L147: Returning to the original submission, the critique of conditional language, here and elsewhere. For example, this can be re-written: “Rates of respiration and methanogenesis in soils and riverine sediments are positively related to temperature suggesting warmer conditions in the lowland rivers contributed to higher pCO2 and dissolved CH4 concentration values. Indeed, pCO2 and dissolved CH4 concentrations increased exponentially with water temperature (Fig. 2d,h), indicating water temperature explains the decreasing pattern of pCO2 and dissolved CH4 concentration with elevation”.

Reply: The text was modified accordingly.

L152: what does ‘marked’ mean? Measurable? Statistically significant?

Reply: We deleted to the word “marked”.

L281: Is there an extra 0 in the elevation?

Reply: The reviewer is absolutely right; typo corrected.

L328: maybe ‘hypothesis’ rather than ‘explanation’? Also, wouldn’t lower k in lowland streams/rivers (on average) lead to greater %N2O?

Reply: The text was modified accordingly. We agree that altitudinal variations of k contribute to gradients in the GHGs. This is discussed in text elsewhere, it seemed unnecessary and possibly distracting to repeat it here.

L418: Seems this sentence refutes including the discussion of the models immediately above it. It might be better to lead with the idea that predicting [GHG] is complex and has high uncertainty, then include some of the variables that others have used in their models to demonstrate the complexity, possible redundancy, and challenges of predicting at global scales. This will also allow you to highlight your approach and why it is different, and perhaps better. Further, the paragraph is also over a page long, I suggest breaking into 2 or more paragraphs.

Reply: The aim of this sentence was not to downplay the models cited above it. Evaluating the added value of increasing the complexity of models is a valid topic, and has been debated in aquatic ecology recurrently during the last decades (*e.g.* Arhonditsis and Brett 2004). It seemed relevant when discussing the validity of our method to mention that there are complex and simple mathematical approaches and that the complex ones are not automatically “better” than more simple ones. Complex mathematical approaches (machine learning based on random forest model) have recently been applied to model river CO_2 emissions. We feel that the community working inland GHG emissions should reflect whether this is the path forward or not. In the present manuscript, based on a data-set in the Amazon basin, we want to make the point that for scaling a handful of data-points simple mathematical models grounded on solid conceptual understanding of the underlying mechanisms can produce useful results. We acknowledge that in future if much larger data-sets are available (we hope so!), probably the mathematical handling of data should be consequently more elaborated.

We have added a sentence to better contextualize.

We followed the reviewer’s recommendation and we have broken down this section into 3 paragraphs.

References

Arhonditsis GB and MT Brett (2004) Evaluation of the current state of mechanistic aquatic biogeochemical modeling, MEPS 271:13-26, doi:10.3354/meps271013