nature portfolio

Peer Review File

Wastewater-influenced estuaries are characterized by disproportionately high nitrous oxide emissions but overestimated IPCC emission factor

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

17th Apr 23

Dear Professor Wang,

Your manuscript titled "Significant nitrous oxide emissions but overestimated IPCC emission factors in wastewater-influenced estuaries" has now been seen by 2 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, in your revised manuscript you should: 1) consider revising the overarching narrative of your manuscript to better incorporate the two sections – that is the in-situ observations of nitrous oxide in the Pearl River estuary and the global meta-analysis – together so that the overall insights and implications of their study are clearer, 2) provide more data/discussion on direct in-situ indicators of wastewater plants discharge and submarine groundwater discharge in the Pearl River Estuary, as well as associated temporal and spatial variabilities, 3) provide comprehensive method details, including for the meta-analysis and study selection criteria (complete and supply the attached meta-analysis checklists at resubmission). Please note we allow unlimited space for methods, 4) discuss your novel estimates in the context of global marine nitrous oxide emissions.

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 us 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,

Annie Bourbonnais, PhD
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Clare Davis, PhD
Senior Editor
Communications Earth & Environment

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Please refer to our data policies at http://www.nature.com/authors/policies/availability.html.

REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

Review of Dong et al.

This paper presents interesting results exploring the sources of N2O from anthropogenically-impacted estuaries. There are two threads presented in this paper. The first set of results indicates that the Pearl River Estuary is a disproportionately large N2O source, and explores the various underlying factors that drive this high accumulation. The second line of argument uses a meta-analysis of global estuaries to suggest that the IPCC may have over-estimated N2O sources from these regions, based on an incorrect formulation of the N2O – NO3- relationship.

In many ways, these two ideas tell different stories, and I found that they did sit naturally in a single paper. It seems to me that two parallel ideas are presented, neither of which had quite enough detail to be fully satisfying. Even the title of the paper seems rather ambiguous, arguing for both high N2O sources locally, but lower than previously estimated N2O sources at a global scale. The result, I think, is a paper whose main message is not nearly as impactful as it could be.

I would suggest that the authors separate these two stories in separate papers, both of which would be very nice. One paper could describe, in significant detail, the distribution of N2O in the PER, taking advantage of an extensive data set (including molecular data) to fully explore the underlying driving factors. Such a paper would be better suited for a more domain-specific journal (e.g.

Limnology and Oceanogaphy). The second paper, which I think could be more appropriate for a Nature family journal, would focus on the global meta-analysis, but with far more detailed analysis and explanation of methods. Specific comments are given below.

Specific comments:

I find the title not particularly satisfying – there are two messages presented, and they seem somewhat contradictory. I think the authors should focus on presenting one clear overall message in the title.

L. 28 – 29: This seems to be a key message in the paper. I find it's diluted with other arguments presented in the paper. It is nicely articulated on lines 70 – 72, and this, I think, should be the main focus of the paper, further developed in the text.

L. 55 – 60. I think there needs to be a clearer distinction between actual N2O concentrations measured, and those inferred from models of NO3-dependent stoichiometry.

L. 68. Biological saturation of what?

L. 82 and elsewhere. Too many significant figures, I think. I'm pretty sure the authors aren't accurately measuring N2O to the fourth decimal place.

L. 123 (and elsewhere). I think the authors some time jump back and forth between figures, describing something in one figure, before completely describing results in an earlier figure.

Fig. 3h. If DO is a predictor of N2O, shouldn't appear on the x axis (independent variable)?

L. 207 – 209. This is a really key result of the paper, which should be expanded and emphasized with more in-depth analysis.
Line 210. In the main text, there wasn't any specific mention about where the N2O data for the global estuaries came from. The information is in the supplementary table, but would be good in the main text also, I think. It would be good to have more discussion about how the estimate NO3- input values were matched in time / space against the measured N2O data. Were the latter integrated / averaged over some time period, or were they 'snap-shots' in time and space. There isn't enough information to evaluate this. A paper focused on the meta-analysis could spend more time describing these details.

Line 372. More information should be presented in the main text about the michaelis menton modelling used to estimate N2O from NO3 loading. Was the model based on the PRE data then extrapolated to all data from the global measurements? The supplementary method 5 cited in the text also doesn't present this information.

Fig. 3i. Maybe replace 'sample ID' with salinity?

Fig. 6. The small colored dots around the coastline are hard to read off the color scale – they all look about the same to me.

Fig. 8. What are the statistics on the MM fit? It looks to me like you could put a linear trend through

that and explain about as much of the variability. Does the MM fit have a lower root mean square, for example. The answer to that question is critical to the conclusions of the paper (which argues that N2O production levels off at higher NO3 loading.

END OF REVIEW.

Reviewer #2 (Remarks to the Author):

Dong and colleagues present a comprehensive approach coupling biogeochemical and microbiological observations to modelling in order to assess the role of wastewater discharge in N2O dynamics of estuarine systems. In particular, the authors aim to highlight that despite enhanced aquatic N2O emissions from these systems, the corresponding emission factors used for current IPCC assessments might have been underestimated because they do not account for non-linear behavior of N2O production/consumption cycles that are brought about by anthropogenic forcing. The manuscript is very well written and the topic is certainly of relevance since recent observations and models clearly indicate the impelling need of further constraining the emissions of N2O in close-coastal systems. Although recent work has suggested the need of updating current IPCC emission factors for nearshore waters (see Maavara, T, Lauerwald, R, Laruelle, GG, et al. Nitrous oxide emissions from inland waters: Are IPCC estimates too high? Glob Change Biol. 2019; 25: 473– 448. https://doi.org/10.1111/gcb.14504); the work by Dong et al presents the first large-scale assessment in this regard from the aquatic (marine) perspective. Overall it is my opinion that the authors' submission represents solid work that is worth publishing in Nature Communications Earth & Environment. I foresee a significant impact for future observational and modelling studies on future emission trends of N2O at the land-ocean boundaries, where some of the largest uncertainties still exist. Nevertheless, there are a few issues which need to be addressed before consider the manuscript for publication. On the following (see attachment), I elaborate on these aspects upon which my assessment is based.

Review of:

"Significant nitrous oxide emissions but overestimated IPCC emission factors in 2 wastewater-influenced estuaries"

by Dong et al.

General Assessment

Dong and colleagues present a comprehensive approach coupling biogeochemical and microbiological observations to modelling in order to assess the role of wastewater discharge in N_2O dynamics of estuarine systems. In particular, the authors aim to highlight that despite enhanced aquatic N_2O emissions from these systems, the corresponding emission factors used for current IPCC assessments might have been underestimated because they do not account for non-linear behavior of N_2O production/consumption cycles that are brought about by anthropogenic forcing. The manuscript is very well written and the topic is certainly of relevance since recent observations and models clearly indicate the impelling need of further constraining the emissions of N_2O in close-coastal systems. Although recent work has suggested the need of updating current IPCC emission factors for nearshore waters (*see Maavara, T, Lauerwald, R, Laruelle, GG, et al. Nitrous oxide emissions from inland waters: Are IPCC estimates too high? Glob Change Biol. 2019; 25: 473– 448. https://doi.org/10.1111/gcb.14504*); the work by Dong et al presents the first large-scale assessment in this regard from the aquatic (marine) perspective. Overall it is my opinion that the authors' submission represents solid work that is worth publishing in Nature Communications Earth & Environment. I foresee a significant impact for future observational and modelling studies on future emission trends of N_2O at the land-ocean boundaries, where some of the largest uncertainties still exist. Nevertheless, there are a few issues which need to be addressed before consider the manuscript for publication. On the following, I elaborate on these aspects upon which my assessment is based.

Main comments

The crux of the manuscript is the changed N_2O dynamics as a response to anthropogenic perturbation, namely wastewater discharge in estuarine waters. Although previous studies cited by the authors clearly show that the Pearl River Estuary is heavily affected by the presence of wastewater plants, I would have expected to see data/discussion based on direct in-situ indicators of that discharge. In particular I wonder about the temporal variability of the discharge and the residence time of those waters in the estuary. Is the discharge dominant year-round or are there any other processes that affect its variability? Are there areas of the estuary in By looking at the cross-estuary (east-west) variability in the several parameters measured by the authors, it would appear reasonable to also account for potential changes on that distribution during the seasonal cycle If it exists, accounting for this variability might help refining both the temporal and spatial dimensions of the emission factors. Along those lines, I wonder whether the impact of this work could be further enhanced by providing a quantitative estimate on how much the nitrogen load to the estuary would have to be reduced to level off the N_2O emissions (which would be important for management decisions). I suspect the authors have all information on hand to provide such an estimate.

Along those lines, the Pearl River Estuary is known to be influenced by submarine groundwater discharge (SGD; *see e.g. Jianan Liu, Jinzhou Du, Ying Wu, Sumei Liu, Nutrient input through*

submarine groundwater discharge in two major Chinese estuaries: the Pearl River Estuary and the Changjiang River Estuary, Estuarine, Coastal and Shelf Science, 203, 17-28, https://doi.org/10.1016/j.ecss.2018.02.005, 2018). Given that SGD can significantly affect the nutrient budget and stoichiometry of coastal systems, it is important to at least discuss whether/how the observed N_2O dynamics can be influenced by SGD in this particular area. Based on the current data, can we actually differentiate between both processes?

As part of their meta-analysis, the authors opted for using a compilation of 81 estuaries for providing a new global-scale assessment of the effect of nitrogen load on estuarine N_2O emissions. The authors acknowledge that their selection is only part of generally available estuarine data sets, which I suspect has to do with lacking information on nitrogen. While this per se does not affect the credibility of the study, it should be at least discussed as a potential limitation.

Surprisingly I did not find any reference nor discussion on the meaning of the updated emissions from estuarine systems provided in this study in the context of global marine N_2O emissions. Based on the work by Yang and colleagues (*PNAS, 117 (22), 11954-11960, https://doi.org/10.1073/pnas.1921914117, 2020*) as well as Tian and colleagues (*Nature, 586, 248–256, https://doi.org/10.1038/s41586-020-2780-0, 2020*), it is clear that close-coastal systems represent some of the largest uncertainties in N_2O emissions, partially because the confounding -and mostly poorly constrained- effects of anthropogenic perturbations. I invite the authors to discuss and quantitatively evaluate how their novel estimates might contribute to the global estimates mentioned above.

I noticed that the authors opted for including so-called "Supplementary discussions 1 – 4". As I am sure authors understand, including discussion items in this section is not an appropriate practice and I therefore invite the authors to critically evaluate whether this information can be shifted to the main document. My impression is, however, that some of these elements rather belong to the supplementary method information. An example of this is Supplementary discussion 1, which contains lots of details on the analytical approach used for the high-resolution surface water measurements.

Additional comments

Generally speaking the manuscript is well organized and the formatting is mostly adequate. Nevertheless, I spotted several abbreviations not spelled in full and inconsistent spelling of e.g. gene markers (which by convention should be written in italics). I therefore recommend the authors to re-check. Also, I recommend the authors to make sure they consistently write the methods in past tense.

l.42: Consider replacing "microorganism" by "microbial activity"

l.64: Since the methods are located at the end of the manuscript (which I am aware is due to the journal's regulations), the reader will not necessarily understand what is meant after seeing the first sentence of the paragraph. Please add reference to the supplementary information.

l.81: Consider replacing "concentration" by "concentrations"

l.81: "the concentration observed". Perhaps the authors mean "mean concentrations"?

l.82: "Humen (zone 1)". If one reads the methods or sees Fig. 2 first, then it is clear what this means. Otherwise it is not. I suggest rearranging the text with cross-references to the Figure and supplementary to be able to quickly grasp the reasoning behind the separation.

l.101: “Determinants”. I would rather call these “drivers”.

l.152: Consider replacing “concentration” by “concentrations”

l.197: Replace “suggestion” by “estimate”

Figures

Considering that the manuscript is about dynamics across an estuary, visualizing the inshore-sea gradient in salinity is something I would expect to see either in Fig. 1 or 2.

Fig. 3: Dissolved oxygen (and derived variables) should be depicted in the x-axis (i.e. as an independent variable). Also, A property-property plot in which the information about the regression made and its statistics can be seen would be much more useful.

Fig. 4: The x-axis says NO3-N but it should actually be NH4-N. Also, in the legend it should read “upstream”. On line 417 it should read “NO3-N”.

Fig. 5: I suspect this is a copy-paste error since the text seems to belong to Figs. S5-7.

Supplementary

In addition to my comment above, please note that the reference to Fig. S9 in the text regarding the set up for high-resolution measurements should read “Fig. S10”. Beyond this, I think the text and figure regarding the method should be the first and not last in the supplementary.

As part of the method description for high-resolution measurements, the authors mention the response time of their system and results of successful tests. I urge the authors to show this information to make complete (and credible) their method description.

RESPONSES TO REFEREES

We would like to express our sincere gratitude to both the reviewers and the editor for their valuable suggestions, which have greatly contributed to the improvement of this manuscript.

Based on the comments received, we have thoroughly revised the manuscript. We have restructured the manuscript to better incorporate *in situ* monitoring of N_2O emission and the global meta-analysis. Specifically, our study focuses on the influence of wastewater discharge on estuarine N_2O emission. We have made the following two major changes:

(1) We have thoroughly revised the content on N_2O concentrations and fluxes in the Pearl River Estuary. By comparing nutrient input sources and analyzing biochemical processes, we have demonstrated the significant impact of wastewater discharge on N_2O emissions.

(2) We have also thoroughly revised our metadata analysis of global estuaries. More specifically, we investigated the possible approaches to refine the IPCC estimate, aiming at better constraining the contribution of estuarine N_2O emissions to the global budget.

We have also revised the overarching narrative of the manuscript. We trimmed down the content related to the driving mechanisms of N_2O emissions in PRE. Instead, we have expanded on the impact of wastewater discharge on N_2O emissions, provided further descriptions and discussions on the global evidence based on the metadata analysis, and offered a comprehensive explanation of the approaches to refine the IPCC estimate.

All changes we made in this round of revision are marked in yellow in the main text. Please find below our point-by-point responses, with the reviewers' comments highlighted in red.

#Reviewer 1:

Comment:

This paper presents interesting results exploring the sources of N2O from anthropogenically-impacted estuaries. There are two threads presented in this paper. The first set of results indicates that the Pearl River Estuary is a disproportionately large N2O source, and explores the various underlying factors that drive this high accumulation. The second line of argument uses a meta-analysis of global estuaries to suggest that the IPCC may have over-estimated N2O sources from these regions, based on an incorrect formulation of the N2O – NO3- relationship.

In many ways, these two ideas tell different stories, and I found that they did sit naturally in a single paper. It seems to me that two parallel ideas are presented, neither of which had quite enough detail to be fully satisfying. Even the title of the paper seems rather ambiguous, arguing for both high N2O sources locally, but lower than previously estimated N_2O sources at a global scale. The result, I think, is a paper whose main message is not nearly as impactful as it could be.

I find the title not particularly satisfying – there are two messages presented, and they seem somewhat contradictory. I think the authors should focus on presenting one clear overall message in the title.

I would suggest that the authors separate these two stories in separate papers, both of which would be very nice. One paper could describe, in significant detail, the distribution of N2O in the PER, taking advantage of an extensive data set (including molecular data) to fully explore the underlying driving factors. Such a paper would be better suited for a more domain-specific journal (e.g. Limnology and Oceanogaphy). The second paper, which I think could be more appropriate for a Nature family journal, would focus on the global meta-analysis, but with far more detailed analysis and explanation of methods. Specific comments are given below.

Response: Thanks for your valuable comment. We have restructured the manuscript to better incorporate these two sections. Specifically, we have reduced the content related to the driving mechanisms of N_2O emissions in PRE. We have provided further descriptions and discussions on the disproportionately high N_2O emission resulting from wastewater discharge, global evidence from the metadata analysis, and the kinetics explanation of overestimated EF_{5e} estimated by IPCC. Additionally, we have thoroughly explored the priority and possible approaches to refine the IPCC estimate to better constrain the contribution of estuarine N_2O emissions to the global budget. The structure of Results and Discussion is also outlined as follows for your reference. Please see more details in the revised manuscript.

- *Heterogeneous N_2O concentrations and high emission fluxes in PRE*
- *Impacts of wastewater discharge on N_2O emissions in the PRE region*
- *Disproportionately high N_2O emissions from global wastewater-influenced estuaries*
- *N_2O emission factors in wastewater-influenced estuaries are significantly lower than IPCC's estimate*
- *Refining IPCC estimate to constrain global estuarine N_2O emission contribution*

The title is revised into "*Disproportionately high nitrous oxide emissions from wastewater-influenced estuaries and the overestimation of IPCC emission factors*".

Comment:

L. 28 – 29: This seems to be a key message in the paper. I find it's diluted with other arguments presented in the paper (line 28-29 in the previous manuscript: *The enhancement of N_2O emission induced by wastewater nitrogen input widely exists in global estuaries, with N_2O emission factors (EF_{5e}) significantly lower than IPCC's suggestion due to progressive biological saturation*).

It is nicely articulated on lines 70 – 72, and this, I think, should be the main focus of the paper, further developed in the text (line 70-72 in the previous manuscript: *The findings of this study demonstrate the important role that wastewater nitrogen discharge play in shaping estuarine N_2O emissions, signifying the need to revise the emission factor and accordingly the regional and global N_2O inventories*).

L. 207 – 209. This is a really key result of the paper, which should be expanded and emphasized with more in-depth analysis. (line 207-209 in the previous manuscript: *The discrepancy suggests that the current global estuarine N_2O emission factor used by IPCC is overestimated by up to an order of magnitude, especially in estuaries with high wastewater influences*)

Response: Thanks for the valuable suggestion. We have enhanced the discussions on the disproportionately high N_2O emissions observed in estuaries influenced by wastewater discharge. Moreover, we have provided in-depth analysis regarding the overestimated IPCC EF_{5e} values and emphasized the necessity to refine the IPCC estimate to better constrain the contribution of estuarine emissions to the global N_2O budget. We substantially revised the Results and Discussion section and added a new section "*Refining linear approaches by IPCC to constrain global estuarine N_2O contribution*". Please see below some new paragraphs we added to discussion, lines 199-211, 224-305 in the revised manuscript:

"……

Estuaries under increasing anthropogenic stress play an increasingly important role in estimating future global N_2O emissions and assessing climate feedback. Globally, approximately 52% of the total nitrogen load to estuarine and riverine watersheds originates from anthropogenic sources[1], *which aligns with the estimate of 56% (based on the ratio of anthropogenic to total nitrogen additions from land) in calculating anthropogenic estuarine emissions in global N_2O budget*[2]. *Enhanced nitrogen loading led to an additional 2.6-9.9 Gmol/yr of N_2O-N emission from estuaries and rivers*[1]. *Estuarine N_2O emission estimates are associated with large uncertainties due to human influences interacting with biochemical processes, resulting in poor constrains on both marine and global N_2O budgets. Global N_2O emissions were estimated to be ~17.0 Tg N yr^{-1}* [1,2], *with ~4.2 Tg N yr^{-1} originating from the ocean*[3]. *Furthermore, approximately 20% of these emissions occur in coastal upwelling systems, which occupy less 3% of the ocean area*[3]. *Improving the accuracy of estuarine N_2O emission estimates is necessary to better constrain its contribution to marine and global budgets under anthropogenic perturbations.*

……

The discrepancy suggests that the current global estuarine N_2O emission factor used by IPCC is overestimated by up to an order of magnitude, particularly in estuaries with high wastewater influences. Previous studies have also revealed large uncertainties associated with IPCC's EF_{5e} estimates [1,4]. Mechanistic modeling approaches that kinetically limit the extent of biochemical reaction have been used to estimate the ranges of EFs for estuaries[1]. The results consistently indicate that IPCC's EFs are too high to be universally applied across all estuaries. Kroeze et al. applied the IPCC default EFs to estimate global estuarine N_2O emissions at 100 Gg N yr^{-1} with a TN input of 44 Tg yr^{-1} [5]. However, if we consider the latest TN load estimate of 97.6 Tg yr^{-1} [1], estuarine N_2O emissions would be approximately 220 Gg N yr^{-1} when using IPCC default EFs, significantly higher than the estimate of 60-155 Gg N yr^{-1} from that based on kinetically mechanistic modeling[1,2].

We analyze the relationship between the observed N_2O and NO_3-N concentrations (Table S1) using three models that incorporate biochemical reaction kinetics in N_2O production (Fig. 6). The linear model assumes a 1st order response, in which N_2O concentrations are directly proportional to NO_3-N concentrations[6]. The efficiency loss model describes a power relationship with an exponent (or order) less than one, indicating that N_2O production by the biota increases with NO_3-N availability, but the efficiency of N_2O concentrations relative to NO_3-N concentrations declines[7]. The third model employs Michaelis-Menten uptake kinetics, representing a saturation of N_2O production as the supply of available NO_3-N exceeds biological demand[8].

Globally, the functional relationship between biological N_2O production and NO_3-N concentration was best described by the efficiency loss model (R^2 = 0.49, p-value < 0.05) (Fig. 6a), in which the efficiency of N_2O production decreases with increasing NO_3-N availability. This efficiency loss model is particularly evident in microbial community adapted to chronic loading, showing high flexibility in response to increasing nutrient concentrations and thus a delayed saturation[9]. In European and Asian estuaries (Fig. 6b and c), stronger statistical results were observed for the Michaelis-Menten model with R^2 = 0.42 and 0.28, respectively. These results show that N_2O production initially increases linearly with NO_3-N concentration but then plateaus at around 1600 nmol L^{-1} when NO_3-N concentration exceeds ~3mg L^{-1}, similar to observations in wastewater-influenced inland water[10]. This plateau is mainly attributed to progressive biological saturation in processing nitrogen with increasing DIN load[11,12]. The number of favorable denitrification and nitrification sites at the water-sediment interface remains relatively constant[13], the efficiency of denitrification and nitrification decreases with increasing nitrogen inputs, eventually leading to relatively stable N_2O emissions at high NO_3-N levels.

High saturation estuaries are prevalent along the coasts of West European, Southern Asia, and Eastern Asia, including estuaries such as Clone and Trent estuaries in UK (Fig. 6b), and the Yellow river and Tianjin river estuaries in China (Fig. 6c). Data for European estuaries are primarily from the 1980s and 2000s. In comparison,

for Asian estuaries, especially in rapidly developing countries such as China and India, the predictable increase in nutrient load[14] is expected to maintain high N_2O emissions. In North America and Oceania (Fig. 6d), a linear response of N_2O to NO_3-N fits more effective, indicating that the increase in nitrogen input can linearly translate to elevated N_2O emissions under conditions of low substrate concentration, as observed in estuaries such as in Werribee estuary in Australia and Chesapeake Bay in USA. Additional fitness statistics for the three models in different estuaries are summarized in Table S4.

……

The assumption of a linear response of N_2O-N to NO_3-N by IPCC is not always valid, as demonstrated by the findings discussed earlier. Therefore, it is crucial to develop a global mechanistic model that explicitly captures the spatial and dynamic relationship between substrate availability and N_2O emissions. Such a model would provide a more accurate representation of the complex processes occurring in estuaries.

Maavara et al. developed a mechanistic modeling approach that incorporates water residence time as a key factor in predicting N_2O emissions[1]. This refinement adds an important kinetic dimension to existing N_2O estimates and better constrains the extent of denitrification and nitrification processes. Additionally, considering piecewise EF_{5e} under different nitrogen load conditions could be an alternative approach to improve the estimation of N_2O emission. Hu et al. have shown the potential of using regression models based on bottom-up data to derive global riverine N_2O EFs across continents and climate zones[13]. Albeit with these advancements in models, the combination of monitoring efforts and modeling endeavors remains essential for improving the accuracy of estuarine N_2O emission estimates.

Specifically, it is crucial to reconsider the role of denitrification and its contribution to N_2O emissions in human-influenced estuaries. The assumption made by IPCC that nitrification produces twice as much N_2O as denitrification[15,16] has been a subject of controversy. Recent studies have reported global nitrification and denitrification fluxes in estuaries to be approximately 0.69 Tmol yr^{-1} and 0.66 Tmol yr^{-1}, respectively[1], indicating a higher contribution from denitrification than suggested by IPCC. Observations in various estuaries, such as Humber[17], Clone[18,19], Tianjin[20], Mulan river[4], and Pear river estuaries, have demonstrated intense N_2O production associated with denitrification, particularly under high NO_3-N concentrations as shown in Fig.6.

In the presence of a high DIN load and low oxygen conditions, denitrification can be stimulated[21], and N_2O is not efficiently consumed as an electron acceptor, resulting in a high N_2O: N_2 ratio (higher than 5%) in estuaries influenced by urban wastewater[22]. This phenomenon is common in eutrophic waters and the upper reaches of estuaries[14]. In comparison, in organic-rich environments, denitrification potential is limited by nitrate availability[22]. In the case of PRE, influenced by wastewater discharge, the ratios of DOC:DIN and DOC:NO_3-N are 0.51 ± 0.09 and 1.25 ± 0.29, respectively, much lower than the levels found in global rivers (average DOC:NO_3-N: 55)[23] and

agriculture/forestlands (median DOC:DIN: 10)[13]*. Globally, there is a negative correlation between DOC:NO_3-N and N_2O concentration (R^2 = 0.23, p-value < 0.05) (Fig. 7a) but a positive correlation with EF_{5e} (R^2 = 0.85, p-value < 0.01) (Fig. 7b). Therefore, in addition to nutrient, the organics and the C:N stoichiometry should considered as important variables for estimating N_2O emission, especially in denitrification-dominant estuaries."*

Comment: L. 55 – 60. I think there needs to be a clearer distinction between actual N2O concentrations measured, and those inferred from models of NO3-dependent stoichiometry.

Response: Thanks for your valuable suggestion. We have restructured the Introduction section to make a clear distinction between biogeochemical N_2O emission and the stoichiometry-dependent N_2O budget. Please see line 48-71 in the revised manuscript. The revised paragraphs are also copied below:

"Estuarine N_2O is primary produced through biochemical processes, including nitrification, denitrification, nitrifier denitrification, and dissimilatory nitrate reduction to ammonium (DNRA)[24]*. The production of estuarine N_2O is governed by nutrients, organic carbon, dissolved oxygen, microbial activity, and hydrologic conditions*[25–27]*. In particular, anthropogenic source, especially wastewater discharge, is important source of estuarine nutrient input*[28]*, and has been estimated to account for ~50% of the total nitrogen load to estuarine and riverine watersheds globally*[1,2]*. Discharge from wastewater treatment plants is generally characterized by low carbon-to-nitrogen ratios*[29] *and low chemical oxygen demand to total nitrogen ratios*[30]*, which stimulates denitrification activity through abundant NO_3-N load, leading to a high N_2O: N_2 ratio with limited dissolved organic carbon (DOC) (electron donor) relative to NO_3-N (electron acceptor)*[10,21,22]*.*

To estimate estuarine N_2O emissions, the Intergovernmental Panel on Climate Change (IPCC) uses dissolved nitrate and the emission factor (EF_{5e}), assuming a linear response of N_2O-N to NO_3-N[31]*. However, previous studies suggested large uncertainties in the estimated global estuarine emission when using the current IPCC EF_{5e}* [1,4,32]*. Considering that factors affecting N_2O emission in natural estuaries are different from those influenced by anthropogenic activities*[32,33]*, using a universal EF_{5e} for all estuaries worldwide is questionable*[1,34]*. Besides, the response pattern of N_2O to N load could make a difference simulated by high nutrient input and C:N stoichiometry change for wastewater-influenced estuaries*[1,22]*. Especially, it is unclear whether the further increase in N input can linearly translate to elevated N_2O emission. In urban rivers and lakes, the biological saturation*[35]*, efficiency loss*[7]*, and*

exponential pattern[36] *have occurred due to exceeded nutrient input. It's necessary to re-valuate the response relationship between* N_2O *emission and nutrient concentration, and the applicability of* EF_{5e} *in human-influenced estuaries."*

Comment: L. 68. Biological saturation of what?

Response: Biological saturation related to processing N with increasing N load, which refers to the saturation of N_2O production as the supply of available NO_3-N exceeds biological demand. We have revised this sentence to *"…due to progressive biological saturation of* N_2O *production, i.e., the supply of available nitrate exceeds biological demand, the* EF_{5e} *…"*.Please see line 79-80 in the revised manuscript.

Comment: L. 82 and elsewhere. Too many significant figures, I think. I'm pretty sure the authors aren't accurately measuring N2O to the fourth decimal place.

Response: Thanks for pointing this out. We have rounded all N_2O concentrations to one decimal place.

Comment : L. 123 (and elsewhere). I think the authors some time jump back and forth between figures, describing something in one figure, before completely describing results in an earlier figure.

Response: Thanks for your comment. We believe you are referring to the part that describes drivers of N_2O emission in PRE. We have removed this part from the revised manuscript.

Comment: Fig. 3h. If DO is a predictor of N2O, shouldn't appear on the x axis (independent variable)?

Response: Thanks for your comment. We have revised the figure (see below). Following your advice, this figure would be used to indicate the underlying driving factors of N_2O production in our follow-up study.

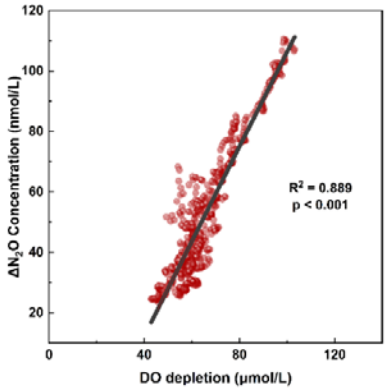

Fig. R1. Linear regression between excess N_2O (ΔN_2O) and oxygen depletion in the middle estuary.

Comment: Line 210. In the main text, there wasn't any specific mention about where the N2O data for the global estuaries came from. The information is in the supplementary table, but would be good in the main text also, I think. It would be good to have more discussion about how the estimate NO3- input values were matched in time / space against the measured N2O data. Were the latter integrated / averaged over some time period, or were they 'snap-shots' in time and space. There isn't enough information to evaluate this. A paper focused on the meta-analysis could spend more time describing these details.

Response: Thank you very much for your valuable comment. We have enriched the metadata analysis and incorporated a more in-depth discussion regarding the spatial correspondence between NO_3-N concentration and N_2O. To illustrate this, we utilized data collected from various continents, which are essentially "snap-shots", and fit three different reaction models. Details regarding this analysis can be found in line 235-268 and are also copied below. Additionally, we discussed the role of denitrification in human-influenced estuaries, and used typical estuaries to support our analysis (see line 285-293). We acknowledge that due to space constraints and the limitations on the number of references, it was challenging to include a supplementary table in the main text outlining the sources of N_2O data for global estuaries. However, we have made every effort to refer to the relevant data tables in the manuscript to provide a clear and concise description.

"We analyze the relationship between the observed N_2O and NO_3-N concentrations (Table S1) using three models that incorporate biochemical reaction kinetics in N_2O production (Fig. 6). The linear model assumes a 1st order response, in which N_2O concentrations are directly proportional to NO_3-N concentrations[6]*. The efficiency loss model describes a power relationship with an exponent (or order) less than one, indicating that N_2O production by the biota increases with NO_3-N availability, but the efficiency of N_2O concentrations relative to NO_3-N concentrations declines*[7]*. The third model employs Michaelis-Menten uptake kinetics, representing a saturation of N_2O production as the supply of available NO_3-N exceeds biological demand*[8]*.*

Globally, the functional relationship between biological N_2O production and NO_3-N concentration was best described by the efficiency loss model (R^2 = 0.49, p-value < 0.05) (Fig. 6a), in which the efficiency of N_2O production decreases with increasing NO_3-N availability. This efficiency loss model is particularly evident in microbial community adapted to chronic loading, showing high flexibility in response to increasing nutrient concentrations and thus a delayed saturation[9]*. In European and Asian estuaries (Fig. 6b and c), stronger statistical results were observed for the Michaelis-Menten model with R^2 = 0.42 and 0.28, respectively. These results show that N_2O production initially increases linearly with NO_3-N concentration but then plateaus at around 1600 nmol L^{-1} when NO_3-N concentration exceeds ~3mg L^{-1}, similar to observations in wastewater-influenced inland water*[10]*. This plateau is mainly attributed to progressive biological saturation in processing nitrogen with increasing DIN load*[11,12]*. The number of favorable denitrification and nitrification*

sites at the water-sediment interface remains relatively constant[13]*, the efficiency of denitrification and nitrification decreases with increasing nitrogen inputs, eventually leading to relatively stable N_2O emissions at high NO_3-N levels.*

High saturation estuaries are prevalent along the coasts of West European, Southern Asia, and Eastern Asia, including estuaries such as Clone and Trent estuaries in UK (Fig. 6b), and the Yellow river and Tianjin river estuaries in China (Fig. 6c). Data for European estuaries are primarily from the 1980s and 2000s. In comparison, for Asian estuaries, especially in rapidly developing countries such as China and India, the predictable increase in nutrient load[14] *is expected to maintain high N_2O emissions. In North America and Oceania (Fig. 6d), a linear response of N_2O to NO_3-N fits more effective, indicating that the increase in nitrogen input can linearly translate to elevated N_2O emissions under conditions of low substrate concentration, as observed in estuaries such as in Werribee estuary in Australia and Chesapeake Bay in USA. Additional fitness statistics for the three models in different estuaries are summarized in Table S4.*

……

Specifically, it is crucial to reconsider the role of denitrification and its contribution to N_2O emissions in human-influenced estuaries. The assumption made by IPCC that nitrification produces twice as much N_2O as denitrification[15,16] *has been a subject of controversy. Recent studies have reported global nitrification and denitrification fluxes in estuaries to be approximately 0.69 Tmol yr^{-1} and 0.66 Tmol yr^{-1}, respectively*[1]*, indicating a higher contribution from denitrification than suggested by IPCC. Observations in various estuaries, such as Humber*[17]*, Clone*[18,19]*, Tianjin*[20]*, Mulan river*[4]*, and Pear river estuaries, have demonstrated intense N_2O production associated with denitrification, particularly under high NO_3-N concentrations as shown in Fig.6.* ”

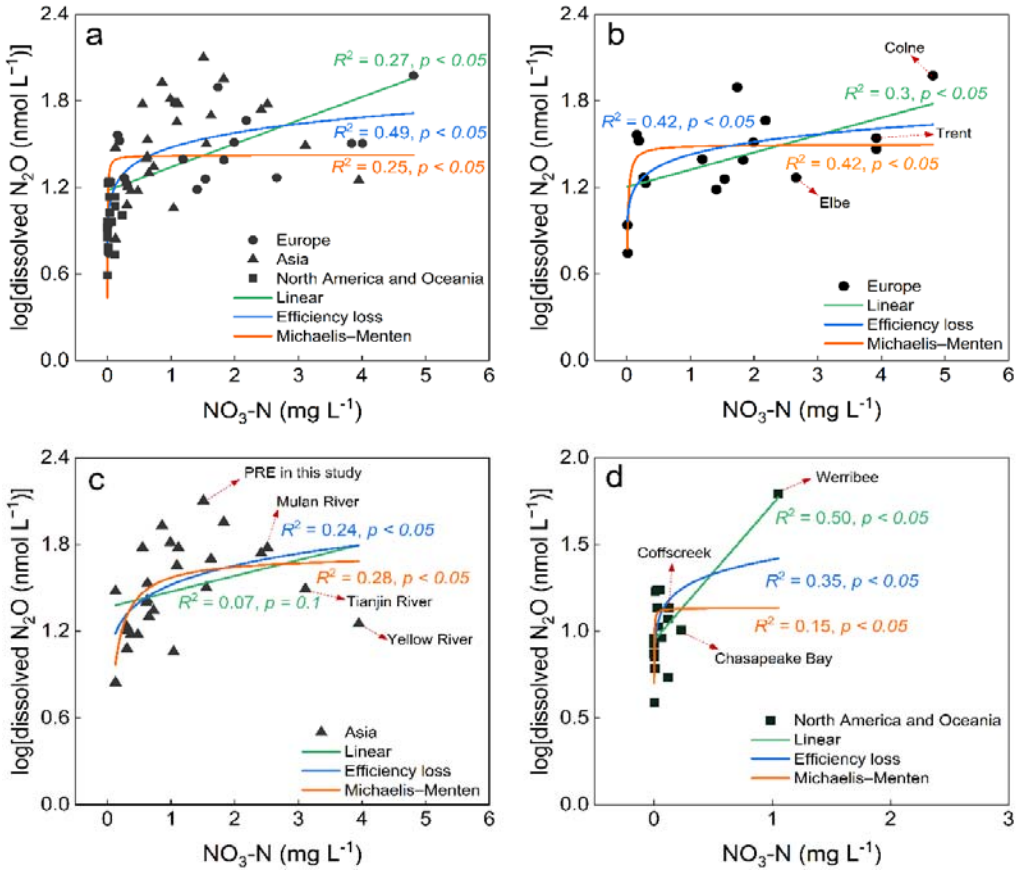

Fig. 6. Linear, efficiency loss, and Michaelis-Menten fit between N_2O concentrations and NO_3-N concentrations for kinetic models in global (a), European (b), Asian (c), North American and Oceanian (d) estuaries. Goodness of fit statistics including residual sum of squares (RSE), adjusted R^2, root mean square error (RMSE), Akaike information criterion (AIC) were shown in this figure and Table S4.

Comment: Line 372. More information should be presented in the main text about the michaelis menton modelling used to estimate N2O from NO3 loading. Was the model based on the PRE data then extrapolated to all data from the global measurements? The supplementary method 5 cited in the text also doesn't present this information.

Response: Thanks for your constructive comment. We collected global estuarine data to conduct Michaelis-Menton modelling. The data used are from previous studies and our research, with details shown in Table S1. We added descriptions for Michaelis-Menton model in the method (line 462-465 and 474-483):

"The linear model, efficiency loss model and Michaelis–Menten kinetics were used to describe the response of dissolved N_2O concentration to nitrate concentration. Estuarine data from previous studies and this study was collected (Table S1) to give a holistic description for the response relationships on a global scale.

……

The Michaelis-Menten kinetics[8] *represents a clear saturation of N2O production as the supply of* NO_3*-N exceeds biological demand. The relationship between* N_2O *concertation and* NO_3*-N concentration is given by:*

$$C = \frac{C_{max} \times [NO_3^-]}{K_m + [NO_3^-]} \quad (10)$$

where C_{max} *represents the maximum* N_2O *concentration achieved in the estuaries, and* K_m *is the required* NO_3*-N concentration to reach half of* C_{max}*.*

In the above regression analyses, dependent variables were log transformed to reduce leverage effects of high N_2O *concentration. We reported residual sum of squares (RSE), adjusted* R^2*, root mean square error (RMSE), Akaike information criterion (AIC) to depict the fitness of different models. Please see details in Supplementary Table S4."*

Comment: Fig. 3i. Maybe replace "sample ID" with salinity?

Response: Thank you so much. We have replotted the figure, which is shown below. Following your advice, this figure would be used to indicate the underlying driving factors of N_2O production in our follow-up study.

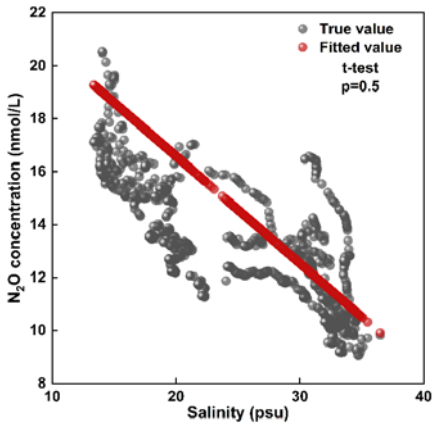

Fig. R2. Fitted N_2O concentration based on a two-end-member mixing model when compared with measurements in the lower estuary

Comment: Fig. 6. The small colored dots around the coastline are hard to read off the color scale – they all look about the same to me.

Response: Thanks for your comment. We added zoom-in view of different continents (Fig. S6 in Supplementary Information) to provide a relatively clear picture of estuarine location and coastline status.

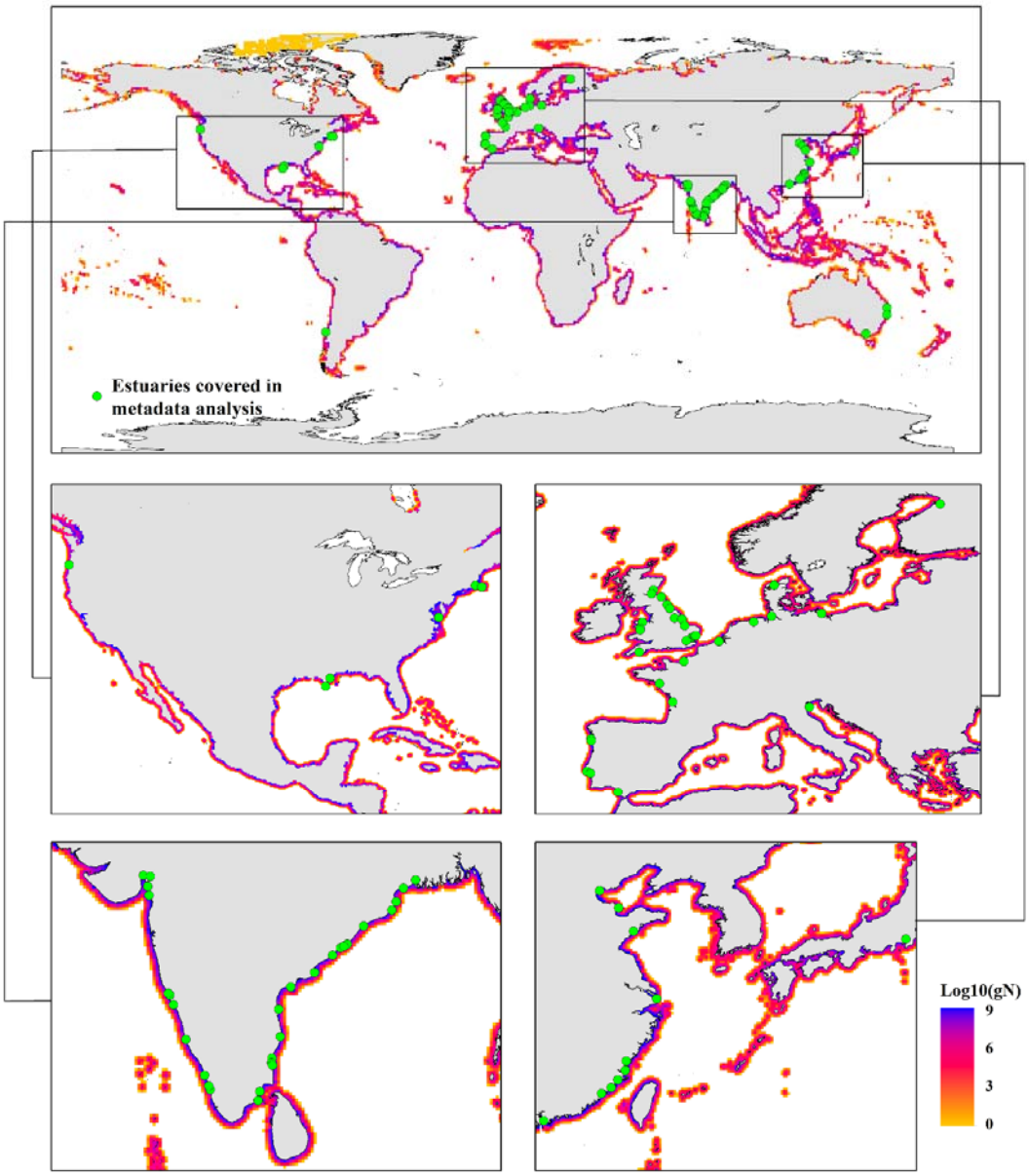

Fig. S6. The distribution of wastewater nitrogen input and estuarine location covered in the metadata analysis.

Comment: Fig. 8. What are the statistics on the MM fit? It looks to me like you could put a linear trend through that and explain about as much of the variability. Does the MM fit have a lower root mean square, for example. The answer to that question is critical to the conclusions of the paper (which argues that N2O production levels off at higher NO3 loading.

Response: Based on your comment, we expanded our analysis by considering reaction kinetics of linear, efficiency loss, and Michaelis-Menten to describe the

relationship between the observed N_2O and NO_3-N concentrations for global estuaries. Detailed method and results are shown in line 235-268 and 461-483. The statistics including residual sum of squares (RSE), adjusted R^2, root mean square error (RMSE), Akaike information criterion (AIC) were used to describe the goodness of fit for different models (Table S4 in the Supporting Information).

Table S4. Goodness of fit for linear, efficiency loss and Michaelis-Menten models in global, Asian, European, American, and Oceania estuaries.

		RSE	Adjusted R^2	p-value	RMSE	AIC
Global	Michaelis-Menten	0.304	0.254	2.547e-41	0.298	32.20
	Linear	0.299	0.274	6.480e-06	0.294	30.50
	Efficiency loss	**0.250**	**0.489**	**1.078e-10**	**0.246**	**8.28**
Asian	**Michaelis-Menten**	**0.269**	**0.281**	**3.367e-19**	**0.258**	**9.43**
	Linear	0.306	0.072	0.100	0.294	16.10
	Efficiency loss	0.277	0.238	0.007	0.266	11.00
European	Michaelis-Menten	0.232	0.423	5.397e-13	0.218	2.46
	Linear	0.256	0.299	0.014	0.240	5.77
	Efficiency loss	**0.233**	**0.419**	**0.003**	**0.219**	**2.57**
American and Oceania	Michaelis-Menten	0.248	0.149	1.216e-10	0.233	4.65
	Linear	**0.1898**	**0.504**	**0.001**	**0.178**	**-4.50**
	Efficiency loss	0.2168	0.351	0.007	0.2038	0.042

#Reviewer 2:

General Assessment

Dong and colleagues present a comprehensive approach coupling biogeochemical and microbiological observations to modelling in order to assess the role of wastewater discharge in N_2O dynamics of estuarine systems. In particular, the authors aim to highlight that despite enhanced aquatic N_2O emissions from these systems, the corresponding emission factors used for current IPCC assessments might have been underestimated because they do not account for non-linear behavior of N_2O production/consumption cycles that are brought about by anthropogenic forcing. The manuscript is very well written and the topic is certainly of relevance since recent observations and models clearly indicate the impelling need of further constraining the emissions of N_2O in close-coastal systems. Although recent work has

suggested the need of updating current IPCC emission factors for nearshore waters (*see Maavara, T, Lauerwald, R, Laruelle, GG, et al. Nitrous oxide emissions from inland waters: Are IPCC estimates too high? Glob Change Biol. 2019; 25: 473– 448. https://doi.org/10.1111/gcb.14504*); the work by Dong et al presents the first large-scale assessment in this regard from the aquatic (marine) perspective. Overall it is my opinion that the authors' submission represents solid work that is worth publishing in Nature Communications Earth & Environment. I foresee a significant impact for future observational and modelling studies on future emission trends of N_2O at the land-ocean boundaries, where some of the largest uncertainties still exist. Nevertheless, there are a few issues which need to be addressed before consider the manuscript for publication. On the following, I elaborate on these aspects upon which my assessment is based.

Response: Thank you very much for your encouraging comments. Please see our responses to your comments below.

Main comments

Comment: The crux of the manuscript is the changed N_2O dynamics as a response to anthropogenic perturbation, namely wastewater discharge in estuarine waters. Although previous studies cited by the authors clearly show that the Pearl River Estuary is heavily affected by the presence of wastewater plants, I would have expected to see data/discussion based on direct in-situ indicators of that discharge. In particular I wonder about the temporal variability of the discharge and the residence time of those waters in the estuary. Is the discharge dominant year-round or are there any other processes that affect its variability? Are there areas of the estuary in By looking at the cross-estuary (east-west) variability in the several parameters measured by the authors, it would appear reasonable to also account for potential changes on that distribution during the seasonal cycle If it exists, accounting for this variability might help refining both the temporal and spatial dimensions of the emission factors. Along those lines, I wonder whether the impact of this work could be further enhanced by providing a quantitative estimate on how much the nitrogen load to the estuary would have to be reduced to level off the N_2O emissions (which would be important for management decisions). I suspect the authors have all information on hand to provide such an estimate.

Along those lines, the Pearl River Estuary is known to be influenced by submarine groundwater discharge (SGD; *see e.g. Jianan Liu, Jinzhou Du, Ying Wu, Sumei Liu, Nutrient input through submarine groundwater discharge in two major Chinese estuaries: the Pearl River Estuary and the Changjiang River Estuary, Estuarine, Coastal and Shelf Science, 203, 17-28, https://doi.org/10.1016/j.ecss.2018.02.005, 2018*). Given that SGD can significantly affect the nutrient budget and stoichiometry of coastal systems, it is important to at least discuss whether/how the observed N_2O dynamics can be influenced by SGD in this particular area. Based on the current data, can we actually differentiate between both processes?

Response: Thank you very much for your valuable comment. The hydrodynamic process of typical wastewater treatment plant N discharge was simulated based on one-dimensional water quality model in PRE. We selected three representative wastewater treatment plants located upstream (Table R1 and Table R2) and simulated the variability of TN based on a simple one-dimensional water quality model (Method R1). The results are shown in Fig. R3 below. Simulated TN concentration exhibited distinct patterns between dry and wet seasons. In the wet season, the larger discharges were accompanied by quicker diffusion and dispersion processes, resulting in lower initial TN concentrations at the same emission intensity level. Due to the higher advection, the pollutant degradation with distance was less pronounced in wet season than in dry season (Fig. R3). While slower velocity and longer residence time in the dry season led to faster TN decrease rates, it is important to highlight that the distribution of TN concentrations within the estuary (which spans a north-south length of approximately 49 km in PRE) was significantly higher compared to the wet season. Consequently, these higher concentrations make a substantial contribution to estuarine N_2O emissions. The critical role that discharges played was further substantiated through a comparison of TN concentration changes between XR and NLT (Fig. R3b and c). In NLT, the higher initial TN concentration, along with a longer degradation distance, occurred due to a relatively smaller discharge compared to XR (Table R2), while emission intensities remained comparable (Table R1). Furthermore, when comparing ZKC and XR, where the discharge conditions were similar (Table R2), the distinct TN trends are due to different emission intensities (Fig. R3a and b). In order to offset the approximately 2.7 times larger wastewater emission intensity, a degradation distance/time that is approximately 1.3-1.8 times longer in ZKC compared with XR would be needed.

Table R1 Representative wastewater treatment plant and discharge details

Wastewater treatment plant	Location	Concentration of TN (mg/L)	Flow (m^3/h)	Emission intensity (mg/s)	Source
ZKC (Guangzhou Zhongke Cheng Sewage Purification Co., LTD)	22.826, 113.549	4.42	4814.5	5911.1	37
XR (Dongguan Xingrun Water Co., Ltd)	22.845, 113.627	9.82	800	2182.2	
NLT (Zhongshan Nanlang Town Water Co., LTD)	22.530, 113.568	8.7	1072	2590.7	

Table R2 Hydrology parameters of estuarine entrances where wastewater treatment plants locate

Wastewater treatment plant	Estuarine entrance	Discharge (m^3/s)		Velocity (m/s)	
		Dry season	Wet season	Dry season	Wet season
ZKC (Guangzhou Zhongke Cheng Sewage Purification Co., LTD)	Humen	1279	6806	0.19	0.49
XR(Dongguan Xingrun Water Co., Ltd)					
NLT (Zhongshan Nanlang Town Water Co., LTD)	Hengmen	214	786	0.14	0.43

Note: all hydrology parameters of estuaries were collected from national hydrology yearbook[38].

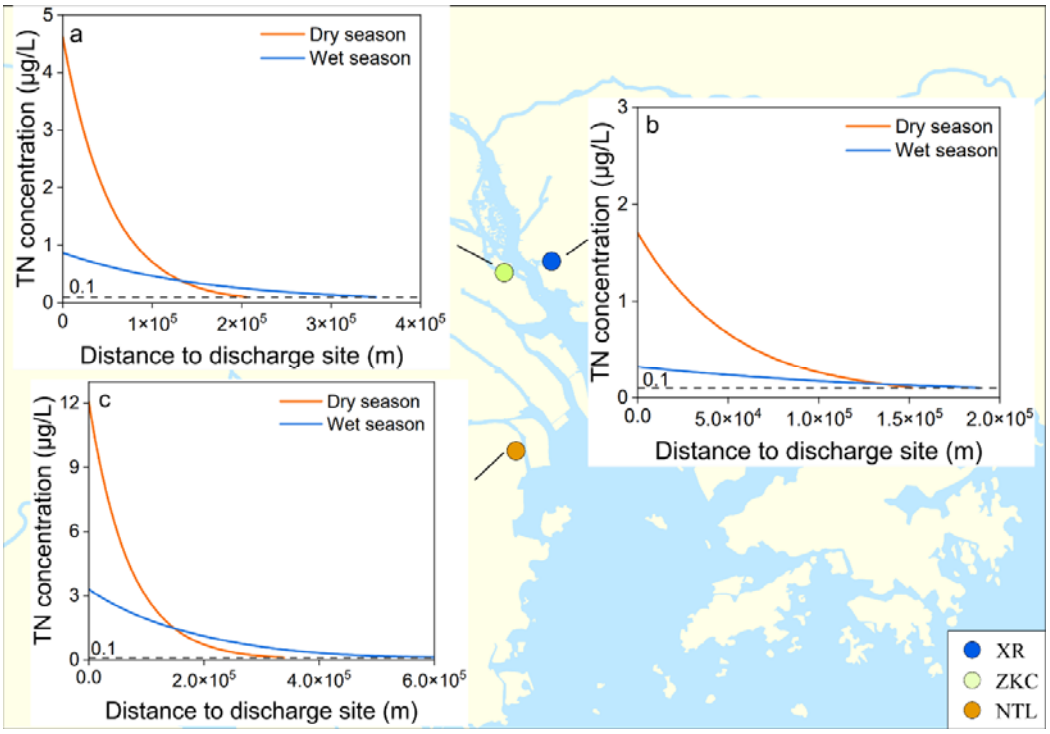

Fig. R3 TN variation trend of wastewater treatment plants (a. ZKC, b. XR, c. NTL) discharge in dry and wet seasons base on one-dimensional water quality model in PRE. Dashed line denotes that TN concentration falls below a limit of 0.1μg/L.

Method R1 Hydrodynamic processes simulation of wastewater N discharge in PRE

Based on mass balance, for constant release, the one-dimensional fully mixed water quality[39] for estuary can be described as:

$$D_x \frac{\partial^2 C}{\partial x^2} - u_x \frac{\partial C}{\partial x} - KC + S_p = 0 \quad \text{(Eq. R1)}$$

where D_x is the mixing and dispersion coefficient in the *x*-direction (m^2/s), u_x is the advection velocity (m/s), K is the rate of pollutant degradation (here the pollutant degradation is TN). S_p is external input. If $C(0) = C_0$, the solution of Eq. R1 is:

$$C = C_0 \exp\left[\frac{u_x x}{2D_x}\left(1 - \sqrt{1 + \frac{4KD_x}{u_x^2}}\right)\right] \quad \text{(Eq. R2)}$$

where $x > 0$, $K = 0.035$, the unit is 1/d.

C_0 is the TN concentration at $x = 0$, it is calculated as:

$$C_0 = \frac{W}{Q\sqrt{1+\frac{4KD_x}{u_x^2}}} \quad \text{(Eq. R3)}$$

Where W is emission intensity of TN (mg/s) at $x = 0$, Q is flux (m^3/s).

D_x is calculated based on emprical formula:

$$D_x = 63 n u_m R^{5/6} \quad \text{(Eq. R4)}$$

Where n is Manning coefficients, u_m is maximum tidal velocity (m/s), R is hydraulic radius of the estuary.

We have also substantially expanded the data analysis and discussion regarding the impact of wastewater discharge to estuarine nutrient input and the associated N_2O emissions. Specifically, we compared the contributions of various sources to the DIN input of PRE, including submarine groundwater, upstream rivers, and wastewater discharge in both dry and wet seasons. We have delved into the biochemical processes of N_2O production, specifically examining how they are influenced by wastewater discharge. We reorganized the analysis of this part in Section "*Impacts of wastewater discharge on N_2O emissions in the PRE region*", line 129-182.

"*Increasing urban wastewater loading can greatly enhance estuarine N_2O production[4,40]. Surrounding by numerous wastewater treatment plants (Fig. 1b), PRE received 5.55 × 10^8 tons of treated wastewater in 2021[37]. When further accounting for indirect discharge from surrounding cities, this value could reach up to 6.80 × 10^9 tons[41]. Assuming the dissolved inorganic nitrogen (DIN) concentration in wastewater effluent is 15 mg/L, which is the limit of highest discharge standard (Class 1A) of TN for municipal wastewater treatment plant in China (GB18918-2002). DIN input was estimated to be (5.95-72.90) ×10^8 mol. Considering the relatively consistent*

wastewater discharge over time, an estimated of (0.16-2.00) × 10^7 mol d^{-1} of DIN loading was estimated during both dry and wet seasons. In addition to wastewater discharge, nutrient inputs from submarine groundwater and upstream river sources were also significant. Submarine groundwater discharge in PRE was estimated to contribute water flows of (4.5-10) ×10^8 m^3 d^{-1} and (1.2-2.7) ×10^8 m^3 d^{-1} in the dry season and wet season, respectively. These flows resulted in DIN loadings of (1.0-43) ×10^7 mol d^{-1} and (0.26-11) ×10^7 mol d^{-1} of DIN loading during the respective seasons[42]*. For riverine nutrient input, the estimated values for DIN loading were ~8×10^7 mol d^{-1} during the dry season and ~20×10^7 d^{-1} mol during the wet season*[42]*. The contribution of nutrient input from wastewater discharge is comparable to that from submarine groundwater and upstream rivers, playing a vital role in regulating the nutrient budget and stoichiometry in PRE (Fig. 3a). Additionally, the relative high TN/TP ratios observed in the aquatic system are associated with the rapid improvement in wastewater treatment*[43]* and submarine groundwater discharge*[42]*, which can potentially contribute to N_2O emission in the estuary*[44]*.*

The upper section of the PRE exhibited higher concentrations of DOC and DIN (Fig. S4), primarily influenced by the influx of carbon and nitrogen from nearby urban wastewater treatment plants. With 97 centralized wastewater treatment plants in Guangzhou and Dongguan surrounding the area, the upstream of Humen received 175,081 thousand tons of treated wastewater in 2021, resulting in a discharge intensity per unit area more than four times higher than that in the middle and the lower sections[37]*. The increasing DIN loads can lead to the formation of hypoxic and anoxia zones in estuaries*[45]*. Upstream in the PRE, lower dissolved oxygen concentrations were observed (Fig. S5), which can further stimulate denitrification and result in high N_2O: N_2 ratio under high NO_3-N concentration condition*[22] *(Fig. S4). Compared with natural estuaries, the discharge from wastewater treatment plants in human-influenced estuaries exhibits lower carbon-to-nitrogen ratios, potentially leading to different N_2O production rates and yields. N_2O concentrations upstream of Humen (zone 1) showed a negative correlation with the ratio of DOC to NO_3-N (R^2 = 0.681, p-value < 0.001) (Fig. 3b). This correlation suggests a possible link between N_2O emission and the nitrogen reduction process*[10]*. With limited DOC relative to NO_3-N, denitrification slows down or stops at N_2O or NO_2^- instead of fully converting to N_2. It is noteworthy that the average ratio of DOC to NO_3-N upstream of PRE was significantly lower compared to five other subtropical estuaries in China*[4]*, where denitrification is favored during N_2O production. In addition, dissolved N_2O showed a relatively stronger correlation with NO_3-N concentration (R^2 = 0.901, p-value < 0.001) than with NH_4-N concentration (R^2 = 0.220, p-value < 0.05) in the upstream of Human (Fig. 3c and d), indicating a higher contribution of denitrification and DNRA to N_2O production compared with nitrification and nitrifier denitrification*[14]*.*

The presence of abundant ammonia-oxidizing and denitrifying genes in PRE (Fig. 3e) indicates that N_2O production is collectively mediated by nitrifying and denitrifying microorganisms. In general, the total abundance of denitrifying genes (nirK, nirS, narG, and nosZ) was much higher than that of AOA amoA and AOB amoA, with the upstream

section of Humen showing higher gene abundances than the middle and lower sections (Fig. 3e). N_2O concentrations showed a positive correlation with the abundance of denitrifying bacterial nirS and nirK genes in the upstream section (R^2 = 0.74, p-value < 0.05) (Fig. 3f), suggesting the important role of denitrification in the upstream N_2O production[4]. In comparison, N_2O concentrations exhibited a stronger correlation with the abundance of ammonia-oxidizing genes than with that of denitrifying genes in Lingdingyang (Fig. 3g). The contribution of nitrification in the upper and middle sections is also supported by the positive correlations between N_2O concentration and NH_4-N substrate (Fig. 3d), which aligns with observations in other anthropogenically influenced estuaries[21].”

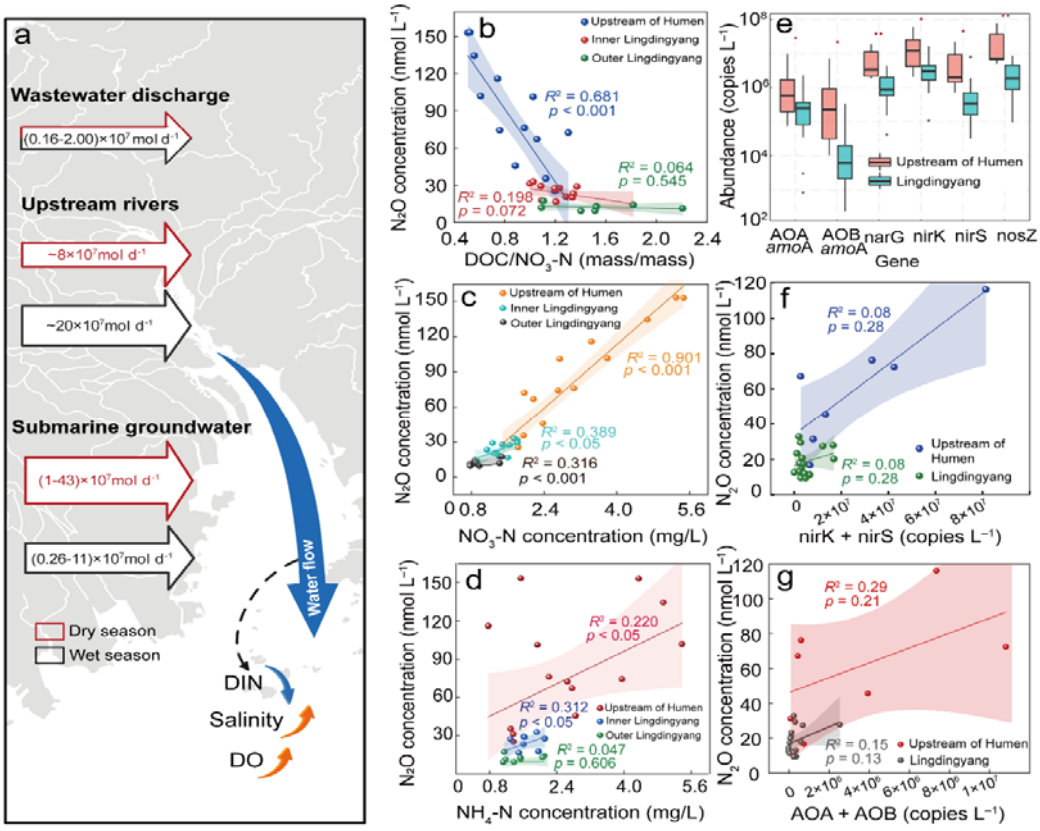

Fig. 3. Wastewater discharge influence and drivers of N_2O emissions in PRE. a, N loading input from wastewater discharge, submarine groundwater and upstream rivers, and a rough spatial variation trend of key physicochemical parameters. Please see detailed spatial distribution of all physicochemical parameters in Fig. S5. **b**, N_2O concentration as a function of DOC/NO_3-N. **c**, Relationship between dissolved N_2O concentration and NO_3-N concentration. **d**, Relationship between dissolved N_2O concentration and NH_4-N concentration. **e,** Abundance distribution and significance of spatial differences of ammonia-oxidizing and denitrification genes in PRE. Symbols “***”, “**”, “*”, and “.” denote different significance levels with *p*-values < 0.001, 0.01, 0.05, and 0.1, respectively. **f**, Linear regression between N_2O concentrations and the abundance of denitrifying genes (*nirS* and *nirK*). **g**, Linear regression between N_2O concentrations and the abundance of ammonia-oxidizing

genes (AOA and AOB *amo*A). Shaded areas in **b, c, d, e, f** and **g** show the 95% confidence bands.

Comment: As part of their meta-analysis, the authors opted for using a compilation of 81 estuaries for providing a new global-scale assessment of the effect of nitrogen load on estuarine N_2O emissions. The authors acknowledge that their selection is only part of generally available estuarine data sets, which I suspect has to do with lacking information on nitrogen. While this per se does not affect the credibility of the study, it should be at least discussed as a potential limitation.

Response: Thanks for your valuable comment. We have added the uncertainties associated with using a limited dataset to estimate global level. Related descriptions are added in line 451-456 and details are listed as follows:

"Using a limited pool of data to generate global estimates inevitably introduces uncertainties. These uncertainties arise due to the highly skewed spatial distribution of the available in situ measurements, which primarily occurred in industrialized countries in Asia, European, and North America, while limited research has been done in Africa and South America. Nevertheless, we included a substantial number of wastewater-influenced estuaries in this study, which improved our understanding of N_2O dynamics in these environments."

Comment: Surprisingly I did not find any reference nor discussion on the meaning of the updated emissions from estuarine systems provided in this study in the context of global marine N_2O emissions. Based on the work by Yang and colleagues (*PNAS, 117 (22), 11954-11960, https://doi.org/10.1073/pnas.1921914117, 2020*) as well as Tian and colleagues (*Nature, 586, 248–256, https://doi.org/10.1038/s41586-020-2780-0, 2020*), it is clear that close-coastal systems represent some of the largest uncertainties in N_2O emissions, partially because the confounding -and mostly poorly constrained- effects of anthropogenic perturbations. I invite the authors to discuss and quantitatively evaluate how their novel estimates might contribute to the global estimates mentioned above.

Response: Thanks very much for your valuable suggestion. We have included a comprehensive discussion regarding the significance of enhancing the accuracy of estuarine N_2O emissions in relation to the marine and global N_2O budget, particularly under anthropogenic perturbations, line 199-211:

" Estuaries under increasing anthropogenic stress play an increasingly important role in estimating future global N_2O emissions and assessing climate feedback. Globally, approximately 52% of the total nitrogen load to estuarine and riverine watersheds originates from anthropogenic sources[1]*, which aligns with the estimate of 56% (based on the ratio of anthropogenic to total nitrogen additions from land) in calculating anthropogenic estuarine emissions in global N_2O budget*[2]*. Enhanced nitrogen loading led to an additional 2.6-9.9 Gmol/yr of N_2O-N emission from estuaries and rivers*[1]*.*

Estuarine N_2O emission estimates are associated with large uncertainties due to human influences interacting with biochemical processes, resulting in poor constrains on both marine and global N_2O budgets. Global N_2O emissions were estimated to be ~17.0 Tg N yr^{-1} [2]*, with ~4.2 Tg N yr^{-1} originating from the ocean*[3]*. Furthermore, approximately 20% of these emissions occur in coastal upwelling systems, which occupy less 3% of the ocean area*[3]*. Improving the accuracy of estuarine N_2O emission estimates is necessary to better constrain its contribution to marine and global budgets under anthropogenic perturbations.* ″

Comment: I noticed that the authors opted for including so-called "Supplementary discussions 1 – 4". As I am sure authors understand, including discussion items in this section is not an appropriate practice and I therefore invite the authors to critically evaluate whether this information can be shifted to the main document. My impression is, however, that some of these elements rather belong to the supplementary method information. An example of this is Supplementary discussion 1, which contains lots of details on the analytical approach used for the high-resolution surface water measurements.

Response: Thanks very much for your valuable comment. We have reorganized the Supplementary Information and moved Supplementary discussion and Supplementary method to the main document. For example, The Supplementary discussion 1 and Supplementary method 1 in the previous manuscript have been merged and moved to the Method: *Fast-Response Automated Gas Equilibrator (FaRAGE) system*, line 359-376 in the revised manuscript:

"The FaRAGE system (Fig. S1) was developed by Xiao et al.[46] *to achieve fast gas–water equilibration, which could be coupled with a greenhouse gas analyzer, making it perfect for field use. The FaRAGE is a flow-through system that adds gas flow into a constant water flow to produce a minimal headspace for continuous concentration measurement. It consists of a gas–water mixing unit and a gas–water separation unit. In the gas–water mixing unit, water samples are continuously pumped to a 50-mL chamber using a peristaltic pump at a rate of 250 mL min^{-1} and are then well-mixed with the carrier gas. Degassing can be rapidly achieved through the jet flow and micro-bubbles generated in the water tube and bubble diffusor and further enhanced in a 2-m long coiled tube (Tygon; inner diameter: 4 mm). The gas–water separation unit then separates headspace gas from water through gravity, and the headspace gas is taken to the greenhouse gas analyzer for measurement. Our tests suggested that the response time of the FaRAGE system (including the response time of the gas analyzer) was 39 ±2 s (Fig. S2). Note that the measured concentration in the headspace was corrected by considering the background N_2O concentration in the carrier gas. The FaRAGE system has been applied to high-frequency, real-time monitoring of N_2O and CH_4 concentrations in the Three Gorges Reservoir*[47,48] *and CH_4 emissions in the upper reaches of the Mekong River*[49]*, enabling accurate mapping of high-resolution gas concentration distribution.* ″

Comment: Generally speaking the manuscript is well organized and the formatting is mostly adequate. Nevertheless, I spotted several abbreviations not spelled in full and inconsistent spelling of e.g. gene markers (which by convention should be written in italics). I therefore recommend the authors to re-check. Also, I recommend the authors to make sure they consistently write the methods in past tense.

Response: Thanks for your kind reminder. We have re-checked the abbreviation, spelling and tense in the whole manuscript. Thanks again.

Comment: l.42: Consider replacing "microorganism" by "microbial activity"

Response: Thank you. Revised, see line 51.

Comment: l.64: Since the methods are located at the end of the manuscript (which I am aware is due to the journal's regulations), the reader will not necessarily understand what is meant after seeing the first sentence of the paragraph. Please add reference to the supplementary information.

Response: Thanks for your comment. Inspired by your suggestion, the method you mentioned was moved from supplementary information to the main text method to improve readability. Thanks again.

Comment 9: l.81: Consider replacing "concentration" by "concentrations"

Response: Thank you for the careful comment. Revised, see line 93.

Comment: l.81: "the concentration observed". Perhaps the authors mean "mean concentrations"?

Response: Thank you for the careful comment. Revised, see line 94.

Comment: l.82: "Humen (zone 1)". If one reads the methods or sees Fig. 2 first, then it is clear what this means. Otherwise it is not. I suggest rearranging the text with cross-references to the Figure and supplementary to be able to quickly grasp the reasoning behind the separation.

Response: Thanks for your kind reminder. We have added cross-references to the Fig. 2a, line 95. Additionally, we double checked the whole manuscript to ensure the appropriate references to Figure, Table and Supplementary Materials to improve readability.

Comment: l.101: "Determinants". I would rather call these "drivers".

Response: Thanks for your comment. Follow the suggestions we received, we have reorganized this part, and the section heading now becomes "*Impacts of wastewater discharge on N2O emissions in the PRE region*". "Determinants" used in the caption of Fig. 3 was revised to "drivers". Please see line 505.

Comment: l.152: Consider replacing “concentration” by “concentrations”

Response: Thank you for the suggestion. Revised, see line 178.

Comment: l.197: Replace “suggestion” by “estimate”

Response: Thank you for the suggestion. Revised, see line 214.

Figures

Comment: Considering that the manuscript is about dynamics across an estuary, visualizing the inshoresea gradient in salinity is something I would expect to see either in Fig. 1 or 2.

Response: Thanks very much for your comment. We have added qualitative description of salinity change in Fig. 2 and added cross reference to Fig. S5 in Supplementation Information, where salinity, Chlorophyll a, dissolved oxygen (DO), oxidation reduction potential (ORP), conductivity, total dissolved solids (TDS), turbidity, pH, and temperature are shown to provide a comprehensive biogeochemical characterization for PRE.

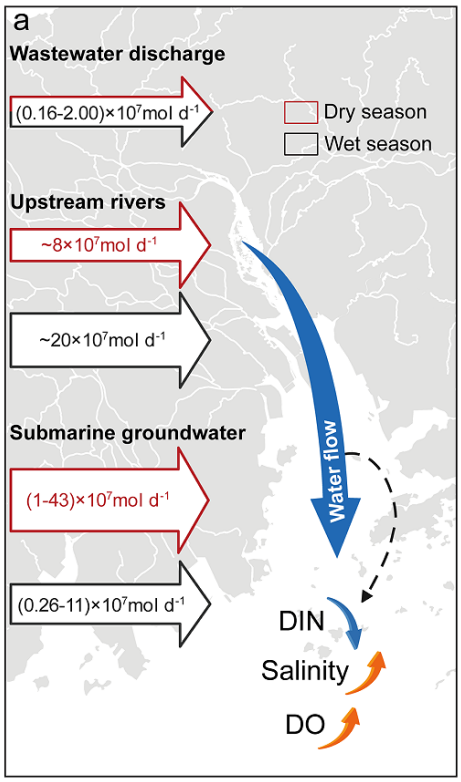

Fig. 3a. N loading input from wastewater discharge, submarine groundwater and upstream rivers, and a rough spatial variation trend of key physicochemical parameters. Please see detailed spatial distribution of all physicochemical parameters in Fig. S5.

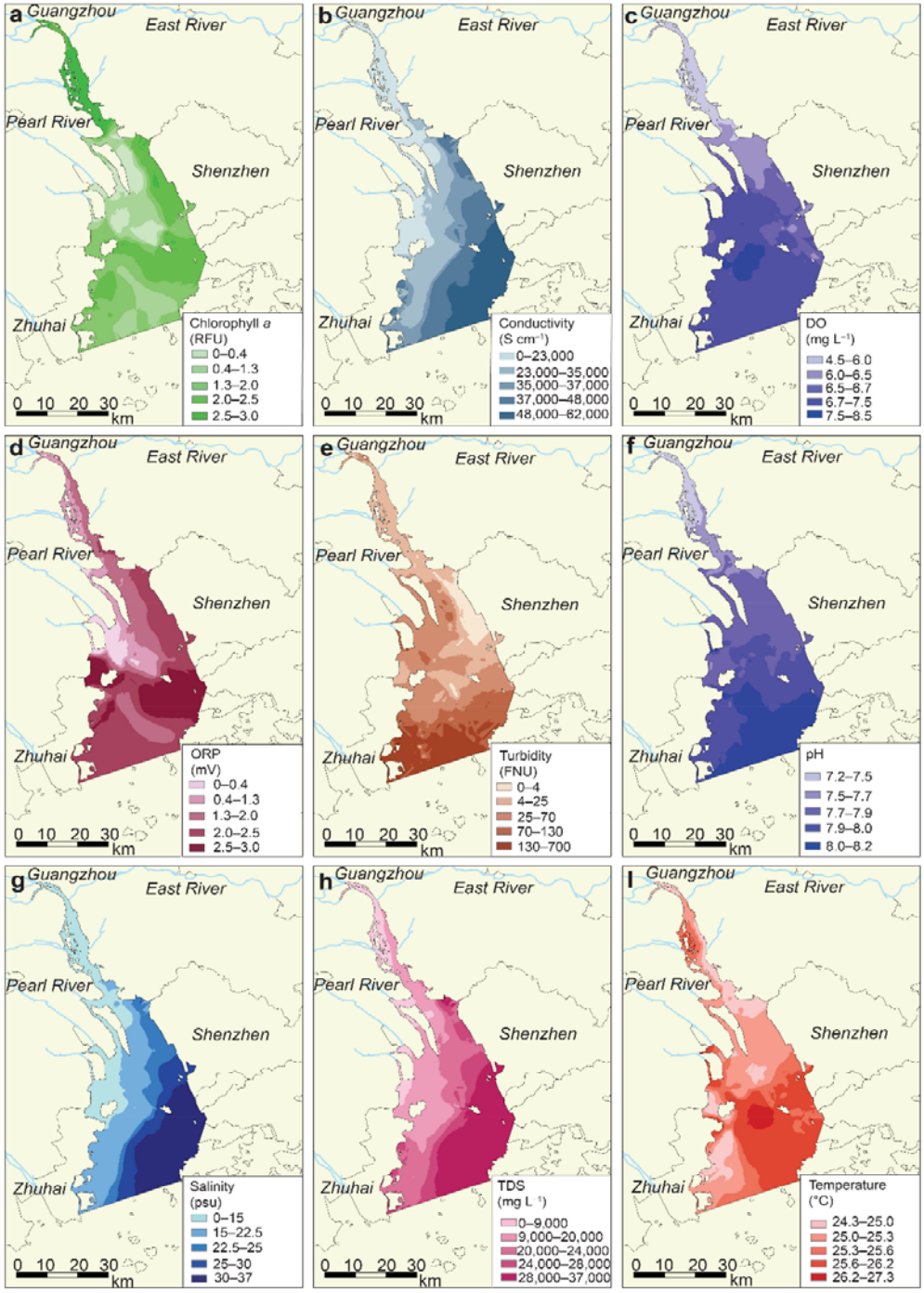

Fig. S5. Spatial distribution of physicochemical parameters. **a**, Chlorophyll a. **b**, Conductivity. **c**, DO. **d**, ORP. **e**, Turbidity, **f**, pH. **g**, Salinity. **h**, Total dissolved solids (TDS). **i**, Temperature.

Comment: Fig. 3: Dissolved oxygen (and derived variables) should be depicted in the x-axis (i.e. as an independent variable). Also, A property-property plot in which the information about the regression made and its statistics can be seen would be much more useful.

Response: Thanks for the suggestion. Please see below a property-property plot. However, considering the comments we received, we have trimmed down the content discussing the underlying driving factors of N_2O production in PRE, and as a result, the corresponding figure has been removed from the manuscript.

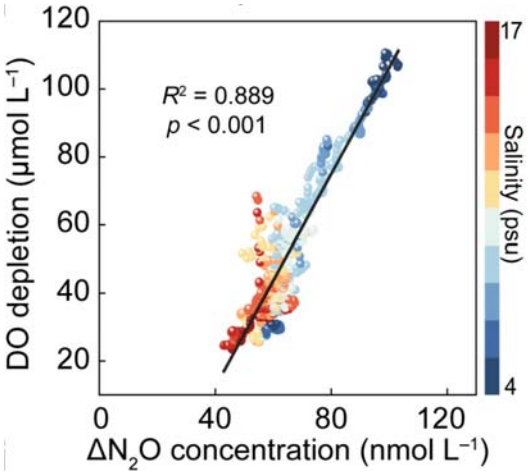

Fig. R4. Linear regression between excess N_2O (ΔN_2O) and oxygen depletion in the middle estuary.

Comment: Fig. 4: The x-axis says NO3-N but it should actually be NH4-N. Also, in the legend it should read "upstream". On line 417 it should read "NO3-N".

Response: Thank you for the comment. We have corrected the Figure and its caption in the revised manuscript.

Comment: Fig. 5: I suspect this is a copy-paste error since the text seems to belong to Figs. S5-7.

Response: Thank you for the comment. We assume that you meant the follow sentence: "Note that symbols "***", "**", "*", and "." denote different significance levels with *p*-values < 0.001, 0.01, 0.05, and 0.1, respectively. Shaded areas in **b** and **c** show the 95% confidence bands."

In Fig. 5a in previous manuscript (Fig. 3e in the revised manuscript), the symbols were applied to demonstrate the different significance level. We have double checked the Figure to make sure it is correct. Thanks again.

Comment:
Supplementary In addition to my comment above, please note that the reference to Fig. S9 in the text regarding the set up for high-resolution measurements should read "Fig. S10". Beyond this, I think the text and figure regarding the method should be the first and not last in the supplementary. As part of the method description for high-resolution measurements, the authors mention the response time of their system and results of successful tests. I urge the authors to show this information to make complete (and credible) their method description.

Response: Thank you for the valuable comment. We have moved the supplementary method you mentioned to the main text. We have also checked the sentence where we cited that figure and ensured it is correct. Additionally, we have added detailed description about the method and added a figure to show the response test of fast-Response Automated Gas Equilibrator (FaRAGE) system. Please see line 359-376 and Fig. S2.

"The FaRAGE system (Fig. S1) was developed by Xiao et al.[46] to achieve fast gas–water equilibration, which could be coupled with a greenhouse gas analyzer, making it perfect for field use. The FaRAGE is a flow-through system that adds gas flow into a constant water flow to produce a minimal headspace for continuous concentration measurement. It consists of a gas–water mixing unit and a gas–water separation unit. In the gas–water mixing unit, water samples are continuously pumped to a 50-mL chamber using a peristaltic pump at a rate of 250 mL min^{-1} and are then well-mixed with the carrier gas. Degassing can be rapidly achieved through the jet flow and micro-bubbles generated in the water tube and bubble diffusor and further enhanced in a 2-m long coiled tube (Tygon; inner diameter: 4 mm). The gas–water separation unit then separates headspace gas from water through gravity, and the headspace gas is taken to the greenhouse gas analyzer for measurement. Our tests suggested that the response time of the FaRAGE system (including the response time of the gas analyzer) was 39±2 s (Fig. S2). Note that the measured concentration in the headspace was corrected by considering the background N_2O concentration in the carrier gas. The FaRAGE system has been applied to high-frequency, real-time monitoring of N_2O and CH_4 concentrations in the Three Gorges Reservoir[47,48] and CH_4 emissions in the upper reaches of the Mekong River[49], enabling accurate mapping of high-resolution gas concentration distribution."

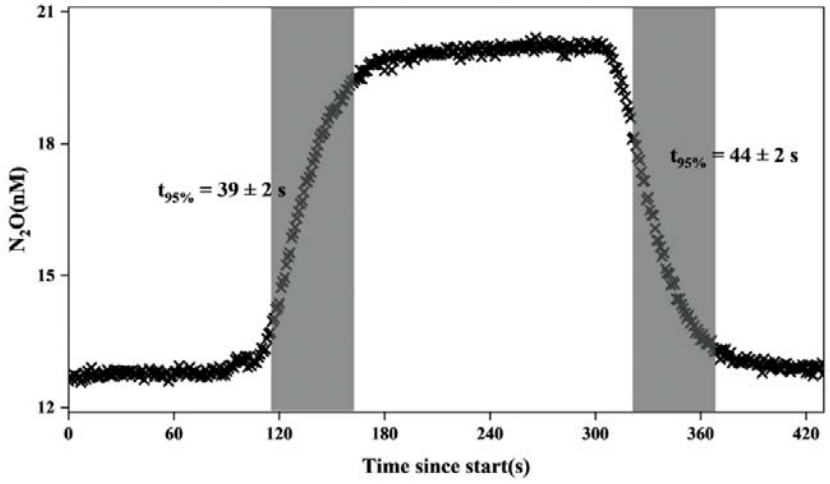

Fig. S2. Response process from the sample entering to the plateaus of measurement with a response time of 39±2s using the FaRAGE-based N_2O measurements

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9th Aug 23

Dear Professor Wang,

Your manuscript titled "Disproportionately high nitrous oxide emissions from wastewater-influenced estuaries and the overestimation of IPCC emission factors" has now been seen by our reviewers, whose comments appear below. In light of their advice we are 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. 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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We hope to hear from you within two weeks; please let us know if you need more time.

Best regards,

Annie Bourbonnais, PhD
Editorial Board Member

Clare Davis, PhD
Senior Editor
Communications Earth & Environment

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REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

The revised manuscript has been streamlined and improved. My major comments have been addressed. However, there are still a number of minor grammatical issues throughout (e.g. missing commas), and these should be addressed prior to final publication.

Reviewer #2 (Remarks to the Author):

I revised an earlier version of this manuscript (with the title: Significant nitrous oxide emissions but overestimated IPCC emission factors in wastewater-influenced estuaries) and despite some concerns I indicated this to be solid work that is suitable for the journal. The issues raised by me during the first round of review were mostly related with missing in-depth analysis of the physical and biogeochemical setting in which the measurements were conducted and how this forcing actually impacts N2O emissions and emission factors.

After revising the authors' response letter and the revised manuscript, it is my opinion that all those concerns have been adequately addressed by including additional data, analysis and discussion. At this point, I only have minor comments which I included in the attached version of the main manuscript and supplementary material. While most of these comments are related to formatting

issues, I would like to emphasize that the comments to the methods section need to be addressed before publication because the current version of the manuscript lacks of clarity in essential aspects of the analytical system used. I don't expect this to affect the study's conclusions, but it is important to ensure that this research is reproducible.

Kind regards,
Damian L. Arévalo-Martínez

Disproportionately high nitrous oxide emissions from wastewater-influenced estuaries and the overestimation of IPCC emission factors

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Abstract

Estuaries are an important contributor to the global nitrous oxide (N_2O) budget. However, considerable uncertainties exist in estuarine N_2O emission estimation due to anthropogenic impacts, primarily wastewater discharge. Here we investigate N_2O emission dynamics in the Pearl River Estuary (PRE), China based on advanced high-resolution, real-time measurements. Our findings suggests that PRE is a strong emission source of N_2O (1.05 Gg yr^{-1}; range: 0.92–1.23 Gg yr^{-1}) with pronounced spatial heterogeneity. Wastewater discharge exerts a substantial influence though introducing abundant nutrients, altering the carbon to nitrogen stoichiometry, and stimulating biochemical processes. The enhancement of N_2O emission induced by wastewater nitrogen input widely exists in global estuaries, with N_2O emission factors (EF_{5e}) considerably lower than that suggested by the IPCC due to progressive biological saturation. Consequently, refining the EF_{5e} estimates with more bottom-up studies is imperative to better constrain the contribution of estuarine emissions to the global N_2O budget.

Introduction

Nitrous oxide (N_2O) is an important stratospheric ozone-depleting substance and the third most important long-lived greenhouse gas (GHG)[1], with a global warming potential 273 times that of CO_2 over a 100-year horizon[2]. As a significant source of N_2O emission to the atmosphere[3], estuaries account for approximately 33% of the global oceanic N_2O emission, despite covering only 0.4% of the world's ocean area[4]. However, N_2O emissions from estuaries remain a poorly constrained term in the global N_2O budget[3]. Many densely populated estuaries receive a substantial amount of wastewater discharged from surrounding urban areas[5]. The wastewater input, together with dynamic anthropogenic–estuarine interactions, leads to strong spatial variations and great uncertainties in estuarine N_2O emission[5,6]. Consequently, Accurately characterizing this spatial heterogeneity requires high-resolution monitoring, which is critical to improving our understanding of regional and global N_2O budgets.

Estuarine N_2O is primary produced through biochemical processes, including nitrification, denitrification, nitrifier denitrification, and dissimilatory nitrate reduction to ammonium (DNRA)[7]. The production of estuarine N_2O is governed by nutrients, organic carbon, dissolved oxygen, microbial activity, and hydrologic conditions[8–10]. In particular, anthropogenic source, especially wastewater discharge, is important source of estuarine nutrient input[11], and has been estimated to account for ~50% of the total nitrogen load to estuarine and riverine watersheds globally[12,13]. Discharge from wastewater treatment plants is generally characterized by low carbon-to-nitrogen ratios[14] and low chemical oxygen demand to total nitrogen ratios[15], which stimulates denitrification activity through abundant NO_3-N load, leading to a high N_2O: N_2 ratio

with limited dissolved organic carbon (DOC) (electron donor) relative to NO_3-N (electron acceptor)[16–18].

To estimate estuarine N_2O emissions, the Intergovernmental Panel on Climate Change (IPCC) uses dissolved nitrate and the emission factor (EF_{5e}), assuming a linear response of N_2O-N to NO_3-N[19]. However, previous studies suggested large uncertainties in the estimated global estuarine emission when using the current IPCC EF_{5e} [12,20,21]. Considering that factors affecting N_2O emission in natural estuaries are different from those influenced by anthropogenic activities[21,22], using a universal EF_{5e} for all estuaries worldwide is questionable[12,23]. Besides, the response pattern of N_2O to N load could make a difference simulated by high nutrient input and C:N stoichiometry change for wastewater-influenced estuaries[12,18]. Especially, it is unclear whether the further increase in N input can linearly translate to elevated N_2O emission. In urban rivers and lakes, the biological saturation[24], efficiency loss[25], and exponential pattern[26] have occurred due to exceeded nutrient input. It's necessary to re-valuate the response relationship between N_2O emission and nutrient concentration, and the applicability of EF_{5e} in human-influenced estuaries.

To address these knowledge gaps, we carried out a high-resolution surface measurement campaign for N_2O concentrations and fluxes in the Pearl River Estuary (PRE), China—a typical wastewater-influenced estuary. We quantify the impact of wastewater discharge on estuarine N_2O emission and identify likely biochemical processes that shape the N_2O dynamics and their spatial heterogeneities in PRE. Through a metadata analysis, we further examine the distinctive patterns of N_2O emission in response to nitrogen input from wastewater in global estuaries. Despite the

relatively small surface area, wastewater-influenced estuaries emit disproportionately high levels of N_2O. Meanwhile, due to progressive biological saturation of N_2O production, i.e., the supply of available nitrate exceeds biological demand, the EF_{5e} in wastewater-influenced estuaries is significantly lower than in undisturbed estuaries and the current IPCC's global level. Findings of this study demonstrate the important role that wastewater nitrogen discharge play in shaping estuarine N_2O emissions, signifying the need to revise the emission factor and accordingly the regional and global N_2O inventories.

Results and Discussion

Heterogeneous N_2O concentrations and high emission fluxes in PRE

Our high-resolution monitoring campaign, consisting of ~30,000 real-time N_2O concentration samples (Fig. 1a and Supplementary Data), reveal strong spatial variations in dissolved N_2O concentrations across the PRE region. The concentrations ranged from 9.1 to 132.2 nmol L^{-1} with an average of 31.7 ± 26.5 nmol L^{-1} (Fig. 2a). The vast majority of PRE exhibited supersaturation in N_2O (140.91–1723.07%) (Fig. 2b), suggesting that the estuary is a net source of atmospheric N_2O[27]. N_2O concentrations in general show an inland-to-sea gradient, with higher mean concentrations observed upstream of Humen (zone 1) at 69.9 ± 27.7 nmol L^{-1}, decreasing to 13.1 ± 2.3 nmol L^{-1} towards the downstream of the estuary (zone 3) (Fig. 2a). The concentrations range in zones 1, 2, and 3 were 30.5–132.2 nmol L^{-1}, 13.0–86.4 nmol L^{-1}, and 9.1–20.7 nmol L^{-1}, respectively, while the corresponding saturation ranges were 415–1723%, 177–1119%, and 141–284%. Along the west-to-east transect, slightly higher N_2O concentrations were observed on the west side (12.8 nmol L^{-1}) compared with the east (9.1 nmol L^{-1}) (transect III). Our high-resolution monitoring also provides detailed insights into the spatial heterogeneity of N_2O concentrations. Notably, we observed a hotspot of N_2O concentration to the northwest of Inner Lingding island (Fig. 2a), where N_2O concentrations reached 25.5 ± 3.9 nmol L^{-1}, significantly higher than the surrounding areas (p-value < 0.001).

Similarly, water–air N_2O flux exhibited substantial spatial variability in PRE, varying by up to two orders of magnitude (5.5–308.3 μmol m^{-2} d^{-1}). The average N_2O flux was 58.5 ± 65.7 μmol m^{-2} d^{-1} (Fig. 2c), which is three times the global estuarine average (18.2 μmol m^{-2} d^{-1};

range: 2.4–199.2 μmol m^{-2} d^{-1})[3]. The average flux density in PRE was comparable to that reported in other human-impacted estuaries, such as Schelde (66.6 μmol m^{-2} d^{-1})[28], Thames (69.1 μmol m^{-2} d^{-1})[29], and Humber (76.6 μmol m^{-2} d^{-1})[30] estuaries in Europe, Werribee estuary (78 μmol m^{-2} d^{-1})[31] in Oceania, and Tokay estuary (77.3 μmol m^{-2} d^{-1})[32] and Adyar estuary (44.3 μmol m^{-2} d^{-1})[33] in Asia.

Our high-resolution monitoring enables reliable N_2O emission estimation. As suggested by previous observations, there are no strong seasonal variations in N_2O concentrations/emissions within the middle and lower estuaries[17]. In comparison, the upstream of Humen has clear seasonal patterns: N_2O concentration and flux in winter and spring are 0.75–2.5 times (on average 1.5 times) higher than in summer and fall[17,24]. Considering the seasonal variability in the upstream of Humen, we estimated an annual emission of 1.05×10^9 (9.22×10^8–1.23×10^9) g N_2O from the entire PRE to the atmosphere, comparable to the total emissions from 19 European inner estuaries[30] (1.35×10^9 g N_2O yr^{-1}; covering an area of ~1840 km^2). Given that the median of annual global N_2O flux[3] is 0.23 (0.13–0.44) Tg, PRE contributes 4.6‰ of the global estuarine N_2O emission with 1.4‰ of global estuarine areas. Especially, the upstream section (zone 1) accounted for up to 42.16% of N_2O emissions with only ~8% of the PRE surface area. When expressed in 100-year global warming potential (GWP), N_2O emission (6.3×10^9 mol yr^{-1} CO_2-equivalent) is equivalent to ~21% of CO_2 emissions (3×10^{10} mol yr^{-1} CO_2)[34] and 161% of CH_4 emission (3.92×10^9 mol yr^{-1} CO_2-equivalent)[35] from PRE. This amount is also close to ~9.3% of the CO_2 sink in coastal systems of the entire China[36].

Impacts of wastewater discharge on N_2O emissions in the PRE region

Increasing urban wastewater loading can greatly enhance estuarine N_2O production[5,20]. Surrounding by numerous wastewater treatment plants (Fig. 1b), PRE received 5.55×10^8 tons of treated wastewater in 2021[37]. When further accounting for indirect discharge from surrounding cities, this value could reach up to 6.80×10^9 tons[38]. Assuming the dissolved inorganic nitrogen (DIN) concentration in wastewater effluent is 15 mg/L, which is the limit of highest discharge standard (Class 1A) of TN for municipal wastewater treatment plant in China (GB18918-2002). DIN input was estimated to be (5.95-72.90) $\times 10^8$ mol. Considering the relatively consistent wastewater discharge over time, an estimated of $(0.16\text{-}2.00) \times 10^7$ mol d^{-1} of DIN loading was estimated during both dry and wet seasons. In addition to wastewater discharge, nutrient inputs from submarine groundwater and upstream river sources were also significant. Submarine groundwater discharge in PRE was estimated to contribute water flows of $(4.5\text{-}10) \times 10^8$ m^3 d^{-1} and $(1.2\text{-}2.7) \times 10^8$ m^3 d^{-1} in the dry season and wet season, respectively. These flows resulted in DIN loadings of $(1.0\text{-}43) \times 10^7$ mol d^{-1} and $(0.26\text{-}11) \times 10^7$ mol d^{-1} of DIN loading during the respective seasons[39]. For riverine nutrient input, the estimated values for DIN loading were $\sim 8 \times 10^7$ mol d^{-1} during the dry season and $\sim 20 \times 10^7$ d^{-1} mol during the wet season[39]. The contribution of nutrient input from wastewater discharge is comparable to that from submarine groundwater and upstream rivers, playing a vital role in regulating the nutrient budget and stoichiometry in PRE (Fig. 3a). Additionally, the relative high TN/TP ratios observed in the aquatic system are associated with the rapid improvement in wastewater treatment[40] and

148 submarine groundwater discharge[39], which can potentially contribute to N_2O emission in the
149 estuary[41].

150 The upper section of the PRE exhibited higher concentrations of DOC and DIN (Fig. S4),
151 primarily influenced by the influx of carbon and nitrogen from nearby urban wastewater treatment
152 plants. With 97 centralized wastewater treatment plants in Guangzhou and Dongguan surrounding
153 the area, the upstream of Humen received 175,081 thousand tons of treated wastewater in 2021,
154 resulting in a discharge intensity per unit area more than four times higher than that in the middle
155 and the lower sections[37]. The increasing DIN loads can lead to the formation of hypoxic and anoxia
156 zones in estuaries[42]. Upstream in the PRE, lower dissolved oxygen concentrations were observed
157 (Fig. S5), which can further stimulate denitrification and result in high N_2O: N_2 ratio under high
158 NO_3-N concentration condition[18] (Fig. S4). Compared with natural estuaries, the discharge from
159 wastewater treatment plants in human-influenced estuaries exhibits lower carbon-to-nitrogen
160 ratios, potentially leading to different N_2O production rates and yields. N_2O concentrations
161 upstream of Humen (zone 1) showed a negative correlation with the ratio of DOC to NO_3-N (R^2 =
162 0.681, p-value < 0.001) (Fig. 3b). This correlation suggests a possible link between N_2O emission
163 and the nitrogen reduction process[16]. With limited DOC relative to NO_3-N, denitrification slows
164 down or stops at N_2O or NO_2^- instead of fully converting to N_2. It is noteworthy that the average
165 ratio of DOC to NO_3-N upstream of PRE was significantly lower compared to five other
166 subtropical estuaries in China[20], where denitrification is favored during N_2O production. In
167 addition, dissolved N_2O showed a relatively stronger correlation with NO_3-N concentration (R^2 =
168 0.901, p-value < 0.001) than with NH_4-N concentration (R^2 = 0.220, p-value < 0.05) in the

upstream of Human (Fig. 3c and d), indicating a higher contribution of denitrification and DNRA to N_2O production compared with nitrification and nitrifier denitrification[14].

The presence of abundant ammonia-oxidizing and denitrifying genes in PRE (Fig. 3e) indicates that N_2O production is collectively mediated by nitrifying and denitrifying microorganisms. In general, the total abundance of denitrifying genes (*nirK*, *nirS*, *narG*, and *nosZ*) was much higher than that of AOA *amo*A and AOB *amo*A, with the upstream section of Humen showing higher gene abundances than the middle and lower sections (Fig. 3e). N_2O concentrations showed a positive correlation with the abundance of denitrifying bacterial *nirS* and *nirK* genes in the upstream section (R^2 = 0.74, p-value < 0.05) (Fig. 3f), suggesting the important role of denitrification in the upstream N_2O production[20]. In comparison, N_2O concentrations exhibited a stronger correlation with the abundance of ammonia-oxidizing genes than with that of denitrifying genes in Lingdingyang (Fig. 3g). The contribution of nitrification in the upper and middle sections is also supported by the positive correlations between N_2O concentration and NH_4-N substrate (Fig. 3d), which aligns with observations in other anthropogenically influenced estuaries[21].

Overall, contributions of abundant nutrient input, low C:N ratios, and relatively low dissolved oxygen levels induced by wastewater discharge collectively stimulate N_2O emissions with stronger denitrification occurring in the upstream section, which intensifies the spatial heterogeneity of N_2O distribution and complicates the biogeochemical processes associated with N_2O emission.

Disproportionately high N_2O emissions from global wastewater-influenced estuaries

To better understand the impact of wastewater discharge on estuarine N_2O emission, we

investigate the nitrogen input from wastewater[43] and N_2O emissions in 83 estuaries globally through a metadata analysis (Table S1, Fig. 4, and Table S2). At the global scale, dissolved N_2O concentrations were positively correlated with wastewater nitrogen input (R^2 = 0.15, p-value < 0.05) (Fig. 5a), suggesting a higher contribution of exogenous nitrogen to N_2O emission in wastewater-influenced estuaries. Based on the level of nitrogen from wastewater discharge, we classified the estuaries into two groups. Compared with estuaries experiencing lighter wastewater influence (nitrogen < 2 Mg N km^{-2}), highly wastewater-influenced estuaries (nitrogen $\geqslant$ 2 Mg N km^{-2}) exhibited significantly higher N_2O flux (p-value < 0.001, t-test) (Fig. 5c), with a median emission level of 12.46 μmol m^{-2} d^{-1} (95% CI: 0.28–76.59 μmol m^{-2} d^{-1}).

Estuaries under increasing anthropogenic stress play an increasingly important role in estimating future global N_2O emissions and assessing climate feedback. Globally, approximately 52% of the total nitrogen load to estuarine and riverine watersheds originates from anthropogenic sources[12], which aligns with the estimate of 56% (based on the ratio of anthropogenic to total nitrogen additions from land) in calculating anthropogenic estuarine emissions in global N_2O budget[13]. Enhanced nitrogen loading led to an additional 2.6-9.9 Gmol/yr of N_2O-N emission from estuaries and rivers[12]. Estuarine N_2O emission estimates are associated with large uncertainties due to human influences interacting with biochemical processes, resulting in poor constrains on both marine and global N_2O budgets. Global N_2O emissions were estimated to be ~17.0 Tg N yr^{-1} [13], with ~4.2 Tg N yr^{-1} originating from the ocean[44]. Furthermore, approximately 20% of these emissions occur in coastal upwelling systems, which occupy less 3% of the ocean area[44]. Improving the accuracy of estuarine N_2O emission estimates is necessary to better constrain its

contribution to marine and global budgets under anthropogenic perturbations.

N_2O emission factors in wastewater-influenced estuaries are significantly lower than IPCC's estimate

We further calculate N_2O emission factors (EF_{5e}) for PRE and 82 other estuaries globally. Across all estuaries, N_2O emission factor is negatively correlated with nitrogen input from wastewater discharge ($R^2 = 0.34$, p-value < 0.05) (Fig. 5b), suggesting a reduced contribution of nitrogen input to water-to-air N_2O diffusive emissions in response to increases nutrient substrate. The N_2O emission factor for PRE is 0.00052 ± 0.00022 kg N_2O-N/kg NO_3-N, while the median EF_{5e} for highly high wastewater-influenced estuaries globally is 0.00099 (95% CI: 0.00011–0.00290) kg N_2O-N/kg NO_3-N. Both values are significantly lower than the IPCC's default value of 0.0026 kg N_2O-N/kg NO_3-N[19]. Even the median EF_{5e} for estuaries with low wastewater influences globally—0.00199 (95% CI: 0.00038 to 0.00754) kg N_2O-N/kg NO_3-N—is lower than the IPCC level. The discrepancy suggests that the current global estuarine N_2O emission factor used by IPCC is overestimated by up to an order of magnitude, particularly in estuaries with high wastewater influences. Previous studies have also revealed large uncertainties associated with IPCC's EF_{5e} estimates [12,20]. Mechanistic modeling approaches that kinetically limit the extent of biochemical reaction have been used to estimate the ranges of EFs for estuaries[12]. The results consistently indicate that IPCC's EFs are too high to be universally applied across all estuaries. Kroeze et al. applied the IPCC default EFs to estimate global estuarine N_2O emissions at 100 Gg N yr^{-1} with a TN input of 44 Tg yr^{-1} [45]. However, if we

consider the latest TN load estimate of 97.6 Tg yr^{-1} [12], estuarine N_2O emissions would be approximately 220 Gg N yr^{-1} when using IPCC default EFs, significantly higher than the estimate of 60-155 Gg N yr^{-1} from that based on kinetically mechanistic modeling[12,13].

We analyze the relationship between the observed N_2O and NO_3-N concentrations (Table S1) using three models that incorporate biochemical reaction kinetics in N_2O production (Fig. 6). The linear model assumes a 1st order response, in which N_2O concentrations are directly proportional to NO_3-N concentrations[46]. The efficiency loss model describes a power relationship with an exponent (or order) less than one, indicating that N_2O production by the biota increases with NO_3-N availability, but the efficiency of N_2O concentrations relative to NO_3-N concentrations declines[25]. The third model employs Michaelis-Menten uptake kinetics, representing a saturation of N_2O production as the supply of available NO_3-N exceeds biological demand[47].

Globally, the functional relationship between biological N_2O production and NO_3-N concentration was best described by the efficiency loss model ($R^2 = 0.49$, p-value < 0.05) (Fig. 6a), in which the efficiency of N_2O production decreases with increasing NO_3-N availability. This efficiency loss model is particularly evident in microbial community adapted to chronic loading, showing high flexibility in response to increasing nutrient concentrations and thus a delayed saturation[48]. In European and Asian estuaries (Fig. 6b and c), stronger statistical results were observed for the Michaelis-Menten model with $R^2 = 0.42$ and 0.28, respectively. These results show that N_2O production initially increases linearly with NO_3-N concentration but then plateaus at around 1600 nmol L^{-1} when NO_3-N concentration exceeds ~3mg L^{-1}, similar to

observations in wastewater-influenced inland water[16]. This plateau is mainly attributed to progressive biological saturation in processing nitrogen with increasing DIN load[49,50]. The number of favorable denitrification and nitrification sites at the water-sediment interface remains relatively constant[51], the efficiency of denitrification and nitrification decreases with increasing nitrogen inputs, eventually leading to relatively stable N_2O emissions at high NO_3-N levels.

High saturation estuaries are prevalent along the coasts of West European, Southern Asia, and Eastern Asia, including estuaries such as Clone and Trent estuaries in UK (Fig. 6b), and the Yellow river and Tianjin river estuaries in China (Fig. 6c). Data for European estuaries are primarily from the 1980s and 2000s. In comparison, for Asian estuaries, especially in rapidly developing countries such as China and India, the predictable increase in nutrient load[3] is expected to maintain high N_2O emissions. In North America and Oceania (Fig. 6d), a linear response of N_2O to NO_3-N fits more effective, indicating that the increase in nitrogen input can linearly translate to elevated N_2O emissions under conditions of low substrate concentration, as observed in estuaries such as in Werribee estuary in Australia and Chesapeake Bay in USA. Additional fitness statistics for the three models in different estuaries are summarized in Table S4.

Refining IPCC estimate to constrain global estuarine N_2O emission contribution

The assumption of a linear response of N_2O-N to NO_3-N by IPCC is not always valid, as demonstrated by the findings discussed earlier. Therefore, it is crucial to develop a global mechanistic model that explicitly captures the spatial and dynamic relationship between substrate

availability and N_2O emissions. Such a model would provide a more accurate representation of the complex processes occurring in estuaries.

Maavara et al. developed a mechanistic modeling approach that incorporates water residence time as a key factor in predicting N_2O emissions[12]. This refinement adds an important kinetic dimension to existing N_2O estimates and better constrains the extent of denitrification and nitrification processes. Additionally, considering piecewise EF_{5e} under different nitrogen load conditions could be an alternative approach to improve the estimation of N_2O emission. Hu et al. have shown the potential of using regression models based on bottom-up data to derive global riverine N_2O EFs across continents and climate zones[51]. Albeit ~~with~~ these advancements in models, the combination of monitoring efforts and modeling endeavors remains essential for improving the accuracy of estuarine N_2O emission estimates.

Specifically, it is crucial to reconsider the role of denitrification and its contribution to N_2O emissions in human-influenced estuaries. The assumption made by IPCC that nitrification produces twice as much N_2O as denitrification[52,53] has been a subject of controversy. Recent studies have reported global nitrification and denitrification fluxes in estuaries to be approximately 0.69 Tmol yr^{-1} and 0.66 Tmol yr^{-1}, respectively[12], indicating a higher contribution from denitrification than suggested by IPCC. Observations in various estuaries, such as Humber[32], Clone[54,55], Tianjin[56], Mulan river[20], and Pear river estuaries, have demonstrated intense N_2O production associated with denitrification, particularly under high NO_3-N concentrations as shown in Fig.6.

In the presence of a high DIN load and low oxygen conditions, denitrification can be stimulated[17], and N_2O is not efficiently consumed as an electron acceptor, resulting in a high N_2O: N_2 ratio (higher than 5%) in estuaries influenced by urban wastewater[18]. This phenomenon is common in eutrophic waters and the upper reaches of estuaries[3]. In comparison, in organic-rich environments, denitrification potential is limited by nitrate availability[18]. In the case of PRE, influenced by wastewater discharge, the ratios of DOC:DIN and DOC:NO_3-N are 0.51 ± 0.09 and 1.25 ± 0.29, respectively, much lower than the levels found in global rivers (average DOC:NO_3-N: 55)[57] and agriculture/forestlands (median DOC:DIN: 10)[51]. Globally, there is a negative correlation between DOC:NO_3-N and N_2O concentration (R^2 = 0.23, p-value < 0.05) (Fig. 7a) but a positive correlation with EF_{5e} (R^2 = 0.85, p-value < 0.01) (Fig. 7b). Therefore, in addition to nutrient, the organics and the C:N stoichiometry should considered as important variables for estimating N_2O emission, especially in denitrification-dominant estuaries.

Taken together, the abundant nutrient loads and altered substrate stoichiometry ratio in highly wastewater-influenced estuaries, such as PRE, are beneficial for N_2O production through nitrification and especially denitrification processes. Consequently, these estuaries often exhibit disproportionately high N_2O emissions, which contribute positively to global warming. However, as the nitrogen load continues to increase, the dissolved N_2O concentration gradually reaches a plateau due to progressive biological saturation. This systematic overestimation of N_2O emission by IPCC is a result of the assumption that N_2O emission linearly responds to nitrogen input. Therefore, it is crucial to refine the relationship between N_2O emission and DIN substrate, and subsequently revise EF_{5e} and global estuarine N_2O emission estimates. This can potentially be

315 achieved through appropriate modeling of the kinetic relationship between carbon and nitrogen
316 substrates with estuarine N_2O emissions. Additionally, comprehensive bottom-up monitoring
317 efforts like the present study are essential to validate and improve the accuracy of the models.
318

Methods

Study area

The Pearl River Estuary (PRE) is located between Guangzhou (23°10' N) and the Wanshan Islands (22°00' N), covering an area of ~1794.6 km^2, where Pearl River, the second largest river in China in terms of annual water discharge (3.3×10^{11} m^3 yr^{-1}), flows into the South China Sea. Surrounded by metropolises such as Hong Kong, Shenzhen, and Guangzhou, this subtropical estuary is substantially influenced by human activities. There are in total 436 centralized wastewater treatment plants, including 199 national key monitoring ones (Fig. 1b), densely scattered around PRE[37,58,59]. The designed capacity of these plants is $23{,}813 \times 10^3$ m^3/d, of which $4{,}942 \times 10^3$ m^3/d is direct discharged into the estuary[37]. In 2021, $554{,}897 \times 10^3$ tons of treated wastewater was discharged into PRE[37]. This value could be up to $6803{,}960 \times 10^3$ tons when further accounting for the indirect discharge from surrounding cities[38]. Human activities (especially wastewater discharge) have led to hypoxia[60] and eutrophication[27] in PRE, which promote high N_2O emissions. With the acceleration of urbanization and industrialization, the N_2O emission from PRE is expected to further increase in the near future.

We carried out field measurements from October 28th to November 1st, 2021. We continuously measured the dissolved N_2O concentrations in the water column during the 5-day cruise. To better understand the spatial variations in N_2O emissions, we divided the entire study area into three zones based on both N_2O concentrations and the geometry of the estuary. Zone 1—upstream of Humen (upper estuary) is from tidal channels in Guangzhou to Humen Outlet, with an area of ~143 km^2. Zone 2—Inner Lingdingyang (middle estuary) is from Humen Outlet

to Inner Lingding Island, with an area of ~848 km^2. Zone 3—Outer Lingdingyang (lower estuary) is from Inner Lingding Island to Outer Estuary, with an area of ~804 km^2.

N_2O concentration measurements

We conducted continuous N_2O concentration measurements using a fast-response automated gas equilibrator (FaRAGE)[61] attached to a gas analyzer (N_2O M1-916; LGR, Canada) on a ship during the 5-day field campaign, which covered the entire PRE (Fig. 1a). The measured N_2O mole fraction was then converted to dissolved concentration in the water column using a device-specific calibration curve, accounting for both partial equilibrations in the device[61] and temperature-dependent solubility[62]. We obtained in total 28,351 individual measurements of dissolved N_2O concentrations, and the sampling locations were recorded by GPS. We then interpolated the spatial distribution using Kriging over the entire PRE.

We also measured multiple physicochemical parameters, including Chlorophyll a, dissolved oxygen (DO), oxidation reduction potential (ORP), salinity, conductivity, total dissolved solids (TDS), turbidity, pH, and temperature, using a multi-parameter probe (EXO2, YSI, USA) (Supplementary Data). In addition, we collected surface water samples at representative sites for biogeochemical and microbial analyses (yellow circles in Fig. 1a). Air temperature, air pressure, and wind speed were measured in situ with a portable anemometer (YGY-QXY). Annual air temperature and precipitation were obtained from the National Meteorological Information Center (http://data.cma.cn/).

Fast-Response Automated Gas Equilibrator (FaRAGE) system

The FaRAGE system (Fig. S1) was developed by Xiao et al.[61] to achieve fast gas–water

equilibration, which could be coupled with a greenhouse gas analyzer, making it perfect for field use. The FaRAGE is a flow-through system that adds gas flow into a constant water flow to produce a minimal headspace for continuous concentration measurement. It consists of a gas–water mixing unit and a gas–water separation unit. In the gas–water mixing unit, water samples are continuously pumped to a 50-mL chamber using a peristaltic pump at a rate of 250 mL min^{-1} and are then well-mixed with the carrier gas. Degassing can be rapidly achieved through the jet flow and micro-bubbles generated in the water tube and bubble diffusor and further enhanced in a 2-m long coiled tube (Tygon; inner diameter: 4 mm). The gas–water separation unit then separates headspace gas from water through gravity, and the headspace gas is taken to the greenhouse gas analyzer for measurement. Our tests suggested that the response time of the FaRAGE system (including the response time of the gas analyzer) was 39±2 s (Fig. S2). Note that the measured concentration in the headspace was corrected by considering the background N_2O concentration in the carrier gas. The FaRAGE system has been applied to high-frequency, real-time monitoring of N_2O and CH_4 concentrations in the Three Gorges Reservoir[63,64] and CH_4 emissions in the upper reaches of the Mekong River[65], enabling accurate mapping of high-resolution gas concentration distribution.

Flux calculation

The N_2O flux through the water–air interface can be estimated based on the following equation

$$F = k_n \times (C_0 - C_e) \quad (1)$$

where F (μmol m^{-2} d^{-1}) is the flux, k_n (cm h^{-1}) is the normalized gas transfer velocity depending on wind and water temperatures, C_0 is the N_2O concentration measured in the water, and C_e is the N_2O concentration in equilibrium with the atmospheric concentration.

The N_2O equilibrium concentration was calculated as[62]

$$C_e = x' K_0 (P - pH_2O) exp\left[P\left(\frac{B+2\delta}{RT} + \bar{v}\left(\frac{1-P}{RT}\right)\right)\right] \quad (2)$$

$$\ln pH_2O = 24.4543 - 67.4509\left(\frac{100}{T}\right) - 4.8489 \ln\left(\frac{100}{T}\right) - 0.000544S \quad (3)$$

where x' is the mole fraction of a constituent of dry air (the global abundance of N_2O was 333.2 ± 0.1 ppb in 2020 according to the Global Atmosphere Watch Programme's in situ observation network; http://www.wmo.int), K_0 is the equilibrium constant (mol L^{-1} atm^{-1}), P is the total pressure, pH_2O is the vapor pressure of water (atm), as a polynomial function of temperature (T) and salinity (S) in eq.(3), $\bar{v}$ is the partial molal volume, R is the gas constant (0.08205601 L atm mol^{-1} K^{-1}), and the term $\frac{B+2\delta}{RT}$ is calculated as

$$\frac{B+2\delta}{RT} = -\frac{9.4563}{T} + 0.04736 - 6.427 \times 10^{-5} T \quad (4)$$

The gas transfer velocity (k) of N_2O was calculated as[66]

$$k = 0.251 \times u_{10}^2 (Sc/660)^{-0.5} \quad (5)$$

where u_{10} is 10-m wind speed, and the temperature-dependent Schmidt number Sc for N_2O was calculated using

$$Sc_{N_2O} = 2141.2 - 152.56T + 5.8963T^2 - 0.12411T^3 \pm 0.0010655T^4 \quad (6)$$

The gas transfer velocity was then normalized to a Schmidt number of 600[67]

$$k_n = k \times (600/Sc)^{-2/3} \quad (7)$$

Note that the 10-m wind speed was derived from wind speed measurements conducted at a height of 4 m[68].

Biogeochemical analyses

Collected water samples were filtered on-site through 0.45 μm polyethersulfone (PES) syringe filters to determine NO_3-N, NO_2-N, NH_4-N, and DOC concentration. The filtrates were collected in 500 ml plastic sampling bottles, and pH was adjusted to < 2 by adding H_2SO_4. The concentrations of NO_3-N, NO_2-N, and NH_4-N were measured by UV spectrophotometry, naphthylamine hydrochloride spectrophotometry, and hypobromite oxidation methods, respectively. DOC concentrations were determined with a TOC analyzer (Shimadzu TOC-5000, Japan) through high-temperature catalytic oxidation. TP concentrations were measured using an ultraviolet–visible spectrophotometer (Agilent 1200) with the standard molybdenum blue method after persulphate digestion.

Microbial analysis

A total of 27 surface water samples collected and filtered through 0.22 μm PES filters were prepared in triplicate for microbial analysis. Particle-associated and free-living communities DNA was extracted from approximately 1.5 L homogenized surface water of filter membrane using an E.Z.N.A. soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). The DNA extracts were checked on 1% agarose gel. DNA concentration and purity were then determined with a NanoDrop 2000 ultraviolet–visible spectrophotometer (Thermo Scientific, Wilmington, USA). After DNA extraction, real-time quantitative polymerase chain reaction (qPCR) was used to estimate the abundances of ammonia-oxidizing (AOA and AOB *amo*A) and denitrifying

bacterial genes (*nirK*, *nirS*, *narG*, and *nosZ*). The abundances of ammonia monooxygenase (AOA and AOB *amo*A) genes were amplified using primer pair sets arch-amoA-23F/arch-amoA-616R and CTO189f/CTO654r. Primer sets cd3aF/R3cd, FlaCu/R3cu, nosZF/nosZR, and narG-f/narG-r, which encompass known diversity of dissimilatory nitrite reductase (*nirS* and *nirK*), nitrous oxide reductase (*nosZ*), and nitrate reductase (*narG*), were used to quantify denitrifying gene abundances. qPCR assays were performed in triplicate on an ABI7300 Thermal Cycler (Applied Biosystems, USA). Detailed information on qPCR primer pairs and amplification conditions is presented in Table S3. A 10-fold dilution series was obtained from extracted plasmid DNA with a known copy number and was used to generate an external standard curve. The amplification efficiencies of the archaeal *amoA*, bacterial *amoA*, *nirS*, *nirK*, *narG*, and *nosZ* genes were 89−104%, and the R^2 values of all amplifications were greater than 0.99.

Global estuarine metadata analysis

To examine the influence of anthropogenic stress on estuarine N_2O emission flux and associated spatial patterns, we investigated the nitrogen input from wastewater[43] and N_2O emissions from estuaries globally through metadata analysis. Estuarine emission data from Asia, Europe, North America, South America and Oceania from 1980s to present were included in the analysis. Dissolved N_2O concentration, N_2O flux, saturation, EF_{5e}, NO_3-N, and DOC: NO_3-N in estuaries we synthesized were summarized in Table S1. For coastal nitrogen distribution from wastewater input, we used a high-resolution (~1 km) global dataset[43], which considers sewered, septic, and untreated wastewater inputs for 142,625 watersheds or coastal areas. The propagation

of nitrogen effluent from each pourpoint into the coastal waters was quantified using a plume model based on a logarithmic decay function[69]. We then extracted wastewater nitrogen input for individual estuaries using ArcGIS 10.4. The delineating boundaries of estuaries we used (irregular polygons) were cross-checked with other studies. We calculated the grid-level statistics (maximum, mean, and standard deviation) of wastewater nitrogen input for each estuary using Python 3.7. The distribution of wastewater nitrogen in 83 estuaries worldwide is shown in Fig. 4 and Fig. S6. Detailed information for the statistics of wastewater nitrogen discharge distribution in these estuaries is summarized in Table S2.

Using a limited pool of data to generate global estimates inevitably introduces uncertainties. These uncertainties arise due to the highly skewed spatial distribution of the available in situ measurements, which primarily occurred in industrialized countries in Asia, European, and North America, while limited research has been done in Africa and South America. Nevertheless, we included a substantial number of wastewater-influenced estuaries in this study, which improved our understanding of N_2O dynamics in these environments.

We performed linear regression, and t-test analysis in R to analyze global estuarine data. Prior to linear regression analysis, we removed outliers based on box plots. The statistical significance of the differences in N_2O concentrations, N_2O fluxes, N_2O saturations, and EF_{5e} was evaluated using *t*-test.

Fit of linear, efficiency loss, and Michaelis-Menten kinetics models

The linear model, efficiency loss model and Michaelis–Menten kinetics were used to describe the response of dissolved N_2O concentration to nitrate concentration. Estuarine data

from previous studies and this study was collected (Table S1) to give a holistic description for the response relationships on a global scale. Linear regression[46] was used to fit the 1st order response of N_2O concentrations to NO_3-N concentrations, described as:

$$C = k \times [NO_3^-] \quad (8)$$

where C represents the N_2O concentration.

In efficiency loss model[25], the N_2O production by biota raises with increasing NO_3-N concentrations, but the efficiency of the N_2O production relative to NO_3-N concentration declines. It was described by a power relationship in which the exponent is less than one:

$$C = k \times [NO_3^-]^n \quad (9)$$

where $n < 1$

The Michaelis-Menten kinetics[47] represents a clear saturation of N_2O production as the supply of NO_3-N exceeds biological demand. The relationship between N_2O concertation and NO_3-N concentration was given by:

$$C = \frac{C_{max} \times [NO_3^-]}{K_m + [NO_3^-]} \quad (10)$$

where C_{max} represents the maximum N_2O concentration achieved in the estuaries, and K_m is the required NO_3-N concentration to reach half of C_{max}.

In the above regression analyses, dependent variables were log transformed to reduce leverage effects of high N_2O concentration. We reported residual sum of squares (RSE), adjusted R^2, root mean square error (RMSE), Akaike information criterion (AIC) to depict the goodness of fit for different models. Please see details in Supplementary Table S4.

Figures

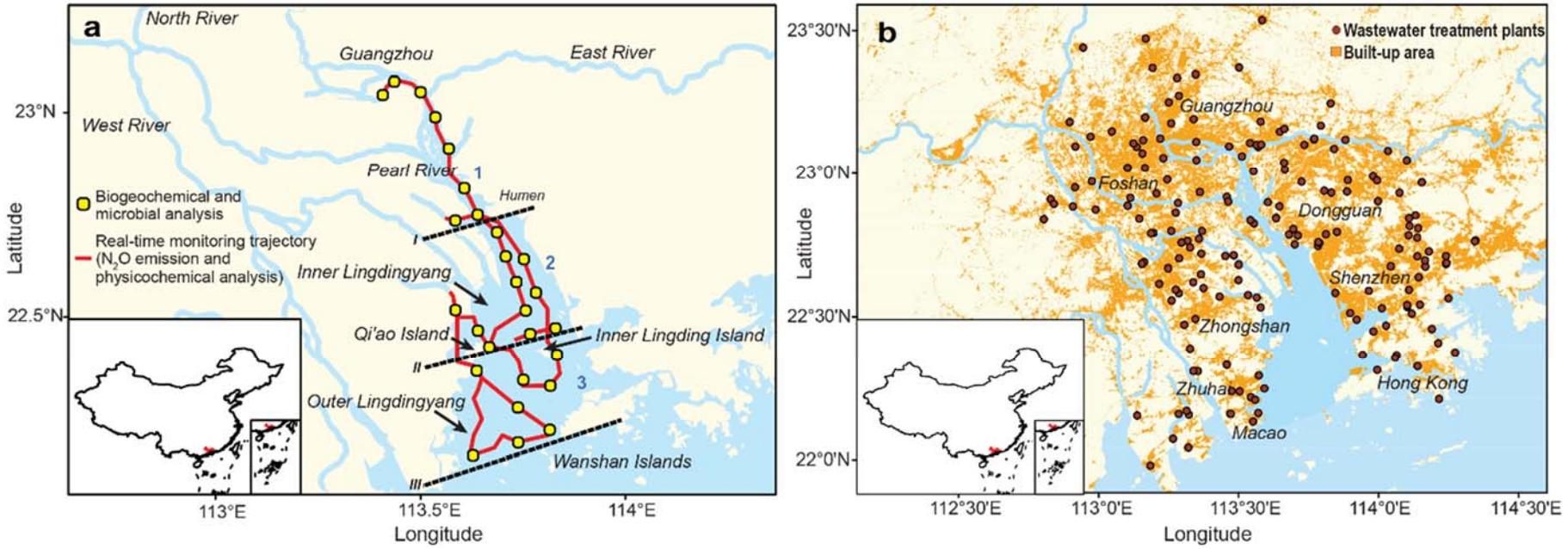


Fig. 1. Real-time high-resolution monitoring trajectory in PRE, China and surrounding wastewater treatment plants. a, Monitoring trajectory and sampling sites. Along the monitoring trajectory (red curve), ~30,000 data points of N_2O concentrations and environmental parameters (Chlorophyll a, dissolved oxygen (DO), oxidation reduction potential (ORP), salinity, conductivity, total dissolved solids (TDS), turbidity, pH, and temperature) were collected. Yellow circles indicate sample sites where biogeochemical parameters (NH_4-N, NO_2-N, NO_3-N, dissolved inorganic nitrogen (DIN), dissolved organic carbon (DOC), and total phosphorus (TP))

and ammonia-oxidizing and denitrifying microbial genes (AOA *amo*A, AOB *amo*A, *nirS*, *nirK*, *nosZ*, and *narG*) with qPCR were analyzed. The entire PRE is partitioned into three zones: (1) Upstream of Humen (upper estuary; zone 1); (2) Inner Lingdingyang (middle estuary; zone 2 between I and II); (3) Outer Lingdingyang (lower estuary; zone 3 between II and III). **b**, Distribution of wastewater treatment plants and built-up areas around PRE. Wastewater treatment plants shown are national key monitoring wastewater treatment plants[25] in Guangzhou, Shenzhen, Dongguan, Foshan, Zhongshan, Zhuhai, and major wastewater treatment plants in Hong Kong[41] and Macao[42]. The inset shows the study area in red on a map of China.

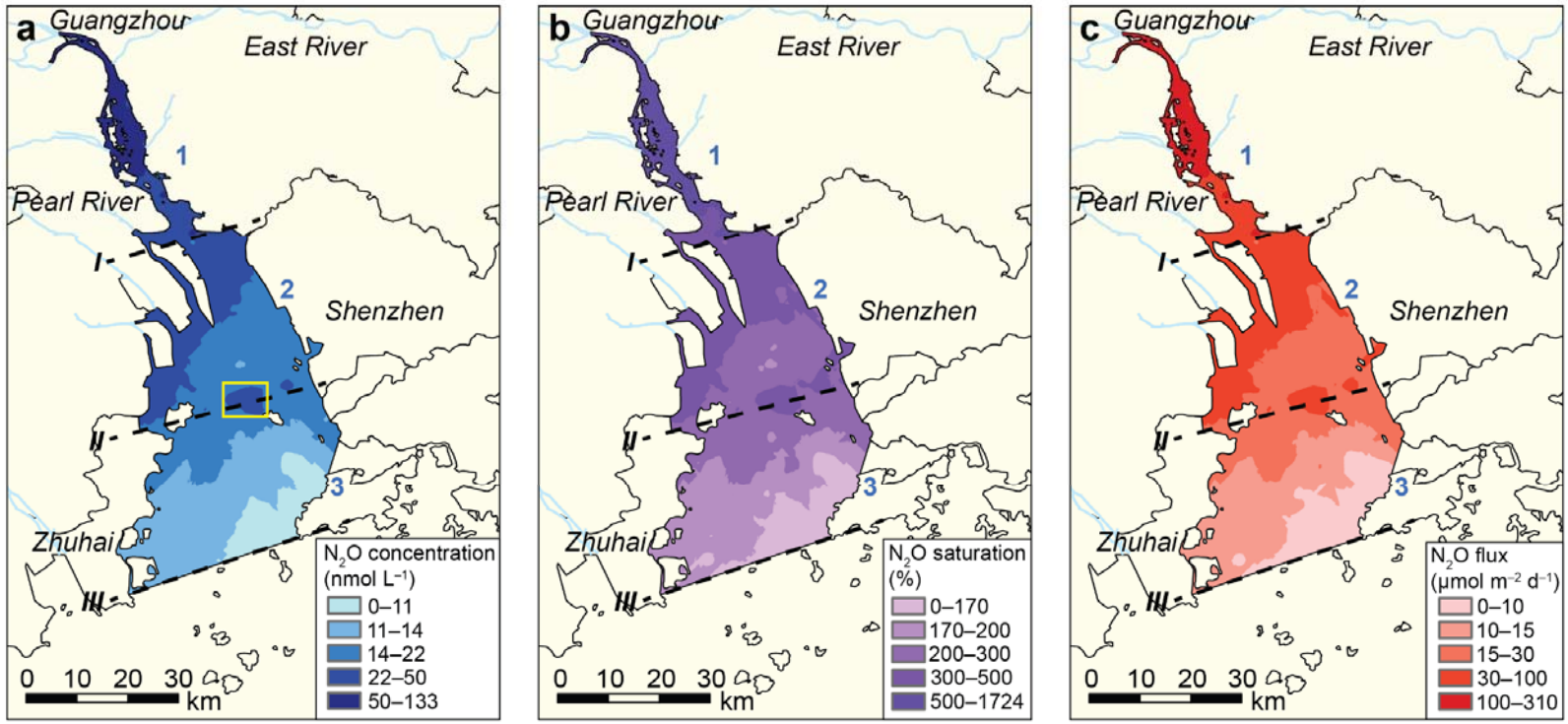
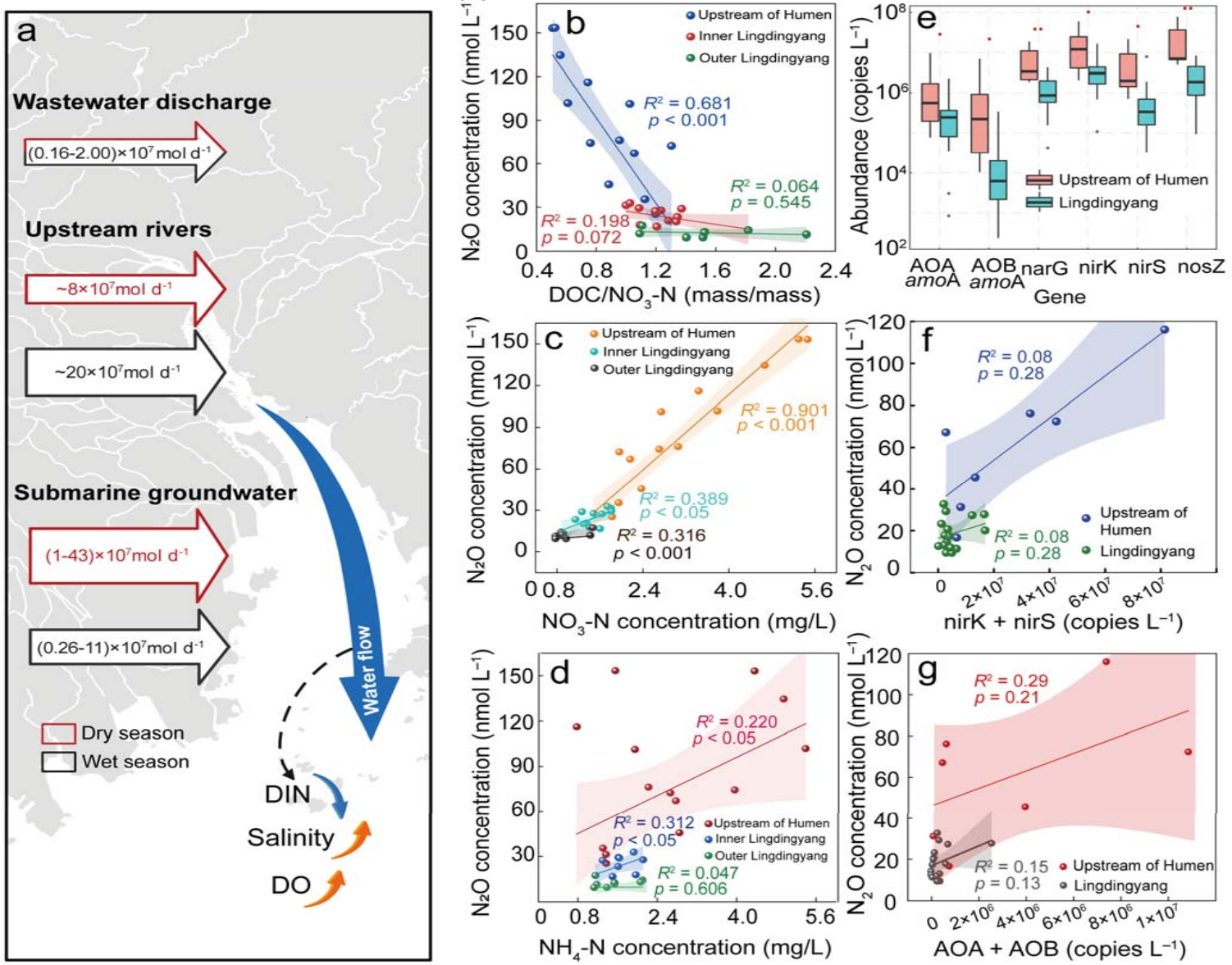
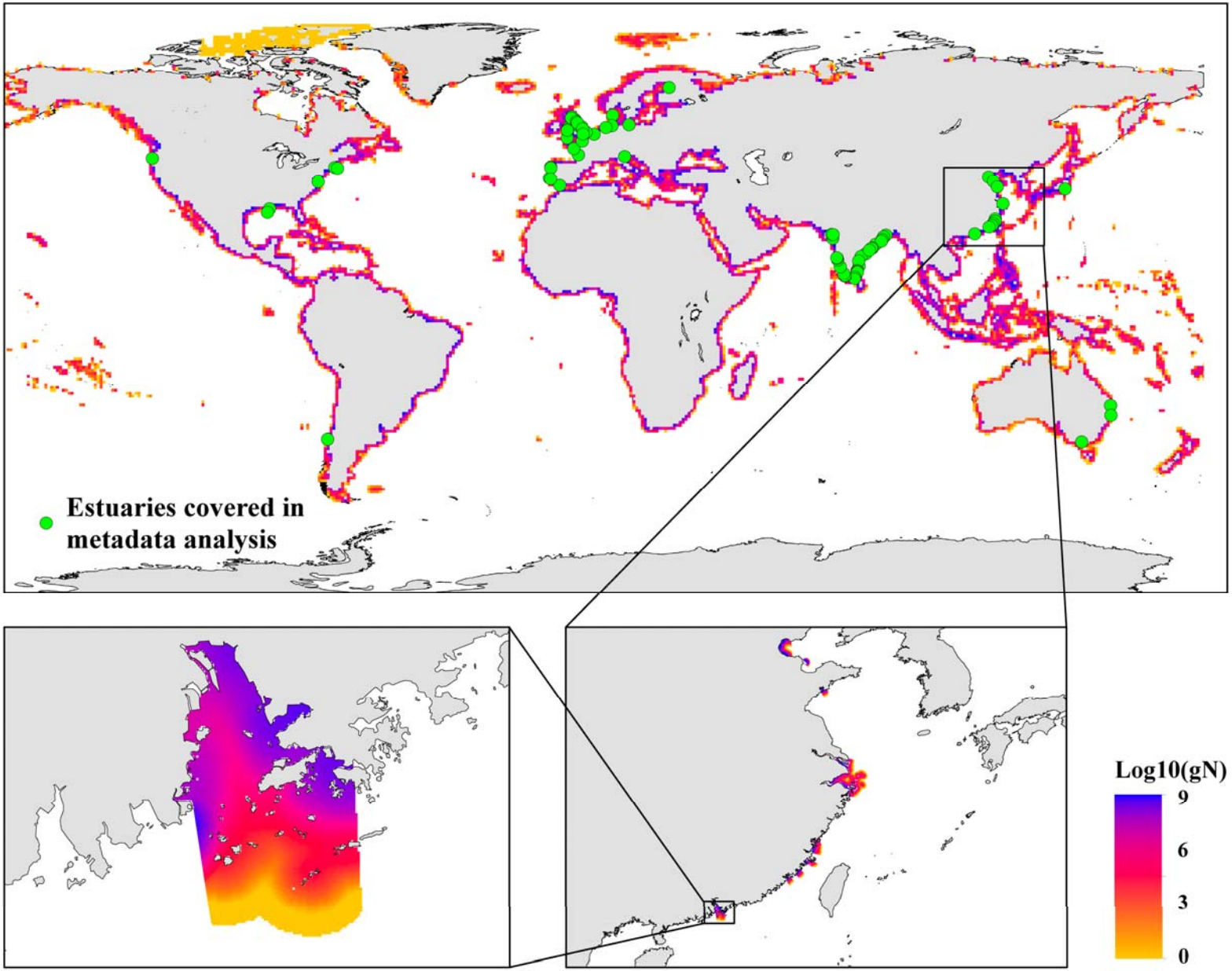


Fig. 2. Spatial distribution of N_2O concentrations, saturations, and fluxes in PRE. a, dissolved N_2O concentrations. **b**, N_2O saturations. **c**, N_2O fluxes. Spatial distributions were interpolated from high-resolution, real-time data (Fig. S3) using Kriging interpolation. The yellow box in **a** indicates the hotspot of N_2O concentration to the northwest of Inner Lingding island.


a
Wastewater discharge
(0.16-2.00)×10⁷ mol d⁻¹
Upstream rivers
~8×10⁷mol d⁻¹
~20×10⁷mol d⁻¹
Submarine groundwater
(1-43)×10⁷mol d⁻¹
(0.26-11)×10⁷mol d⁻¹
Dry season
Wet season
Water flow
DIN
Salinity
DO
b
Upstream of Humen
Inner Lingdingyang
Outer Lingdingyang
R² = 0.681
p < 0.001
R² = 0.064
p = 0.545
R² = 0.198
p = 0.072
N₂O concentration (nmol L⁻¹)
DOC/NO₃-N (mass/mass)
e
Abundance (copies L⁻¹)
Upstream of Humen
Lingdingyang
AOA amoA
AOB amoA
narG
nirK
nirS
nosZ
Gene
c
Upstream of Humen
Inner Lingdingyang
Outer Lingdingyang
R² = 0.901
p < 0.001
R² = 0.389
p < 0.05
R² = 0.316
p < 0.001
N₂O concentration (nmol L⁻¹)
NO₃-N concentration (mg/L)
f
R² = 0.08
p = 0.28
R² = 0.08
p = 0.28
Upstream of Humen
Lingdingyang
N₂O concentration (nmol L⁻¹)
nirK + nirS (copies L⁻¹)
d
R² = 0.220
p < 0.05
R² = 0.312
p < 0.05
R² = 0.047
p = 0.606
Upstream of Humen
Inner Lingdingyang
Outer Lingdingyang
N₂O concentration (nmol L⁻¹)
NH₄-N concentration (mg/L)
g
R² = 0.29
p = 0.21
R² = 0.15
p = 0.13
Upstream of Humen
Lingdingyang
N₂O concentration (nmol L⁻¹)
AOA + AOB (copies L⁻¹)

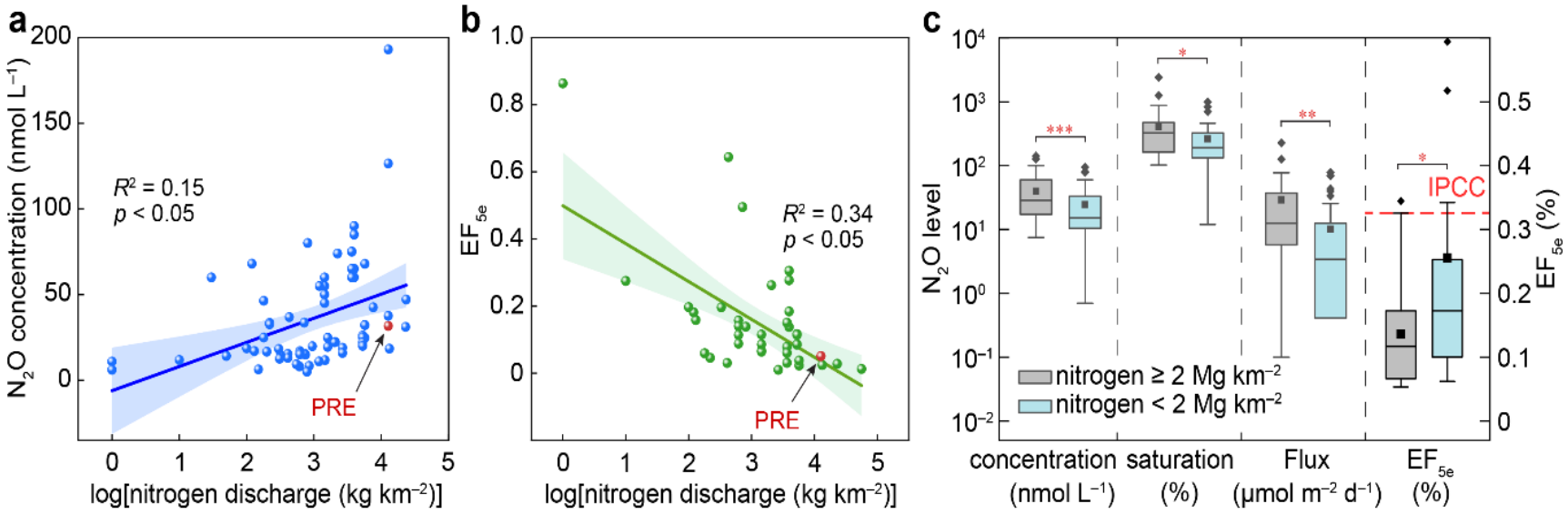
504

Fig. 3. Wastewater discharge influence and drivers of N_2O emissions in PRE. a, N loading input from wastewater discharge, submarine groundwater and upstream rivers, and a rough spatial variation trend of key physicochemical parameters. Please see detailed spatial distribution of all physicochemical parameters in Fig. S5. **b**, N_2O concentration as a function of DOC/NO_3-N. **c**, Relationship between dissolved N_2O concentration and NO_3-N concentration. **d**, Relationship between dissolved N_2O concentration and NH_4-N concentration. **e,** Abundance distribution and significance of spatial differences of ammonia-oxidizing and denitrification genes in PRE. Symbols "***", "**", "*", and "." denote different significance levels with *p*-values < 0.001, 0.01, 0.05, and 0.1, respectively. **f**, Linear regression between N_2O concentrations and the abundance of denitrifying genes (*nirS* and *nirK*). **g**, Linear regression between N_2O concentrations and the abundance of ammonia-oxidizing genes (AOA and AOB *amo*A). Shaded areas in **b, c, d, e, f** and **g** show the 95% confidence bands.



514

Fig. 4. Wastewater nitrogen in global estuaries. a, The distribution of wastewater nitrogen input in all estuaries globally. **b**, Zoomed-in view of estuarine wastewater nitrogen in China. **c**, Zoomed-in view of estuarine wastewater nitrogen in PRE. Wastewater nitrogen input is shown in log10(gN). Green circles in **a** show the 83 estuaries covered in the metadata analysis. Zoom-in view of different continents were shown in Fig. S6 to provide a relatively clear picture of estuarine location and coastline status.



520

521 **Fig. 5. N_2O emissions influenced by wastewater nitrogen discharge in global estuaries. a**, N_2O concentration in relation to

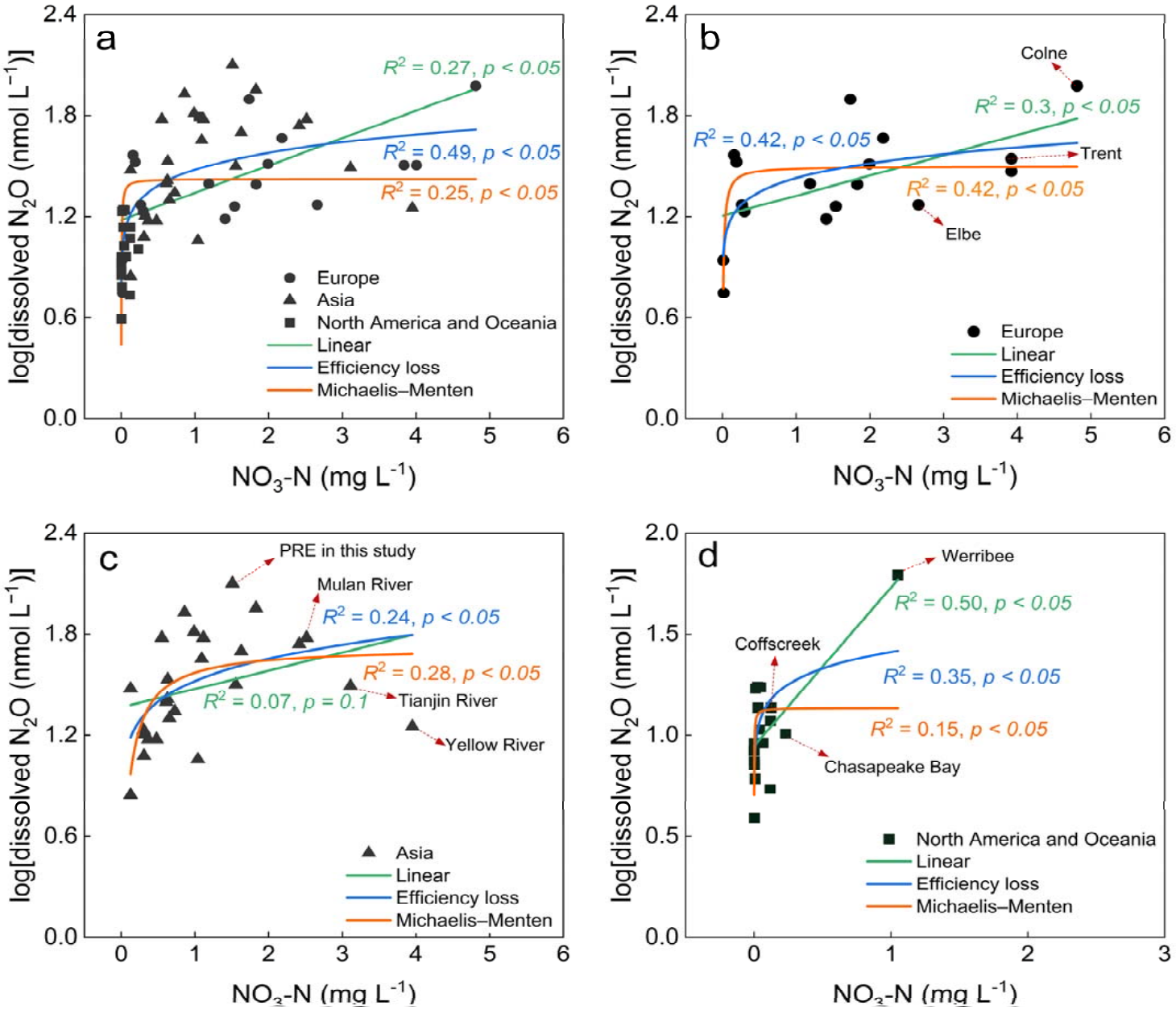
522 wastewater nitrogen discharge, **b**, EF_{5e} in relation to wastewater nitrogen discharge. **c,** Levels of N_2O concentration, saturation, and

523 emission flux and emission factors (EF_{5e}) in two groups of estuaries worldwide with different levels of wastewater nitrogen discharge

524 (estuaries with high human impacts: wastewater nitrogen ≥ 2 Mg N km^{-2}; estuaries with low human impacts: wastewater nitrogen < 2

525 Mg N km^{-2}). Details of estuaries are summarized in Table S1. Symbols "***", "**", and "*" in **c** denote p-values < 0.001, 0.01, and

526 0.05, respectively. Shaded areas in **a** and **b** show the 95% confidence bands.


a
R^2 = 0.27, p < 0.05
R^2 = 0.49, p < 0.05
R^2 = 0.25, p < 0.05
Europe
Asia
North America and Oceania
Linear
Efficiency loss
Michaelis–Menten
log[dissolved N_2O (nmol L^{-1})]
NO_3-N (mg L^{-1})
b
Colne
Trent
Elbe
R^2 = 0.3, p < 0.05
R^2 = 0.42, p < 0.05
R^2 = 0.42, p < 0.05
Europe
Linear
Efficiency loss
Michaelis–Menten
c
PRE in this study
Mulan River
Tianjin River
Yellow River
R^2 = 0.24, p < 0.05
R^2 = 0.28, p < 0.05
R^2 = 0.07, p = 0.1
Asia
Linear
Efficiency loss
Michaelis–Menten
d
Werribee
Coffscreek
Chasapeake Bay
R^2 = 0.50, p < 0.05
R^2 = 0.35, p < 0.05
R^2 = 0.15, p < 0.05
North America and Oceania
Linear
Efficiency loss
Michaelis–Menten

527

Fig. 6. Linear, efficiency loss, and Michaelis-Menten fit between N_2O concentrations and NO_3-N concentrations for kinetic models in global (a), European (b), Asian (c), North American and Oceanian (d) estuaries. Goodness of fit statistics including residual sum of squares (RSE), adjusted R^2, root mean square error (RMSE), Akaike information criterion (AIC) were shown in this figure and Table S4.

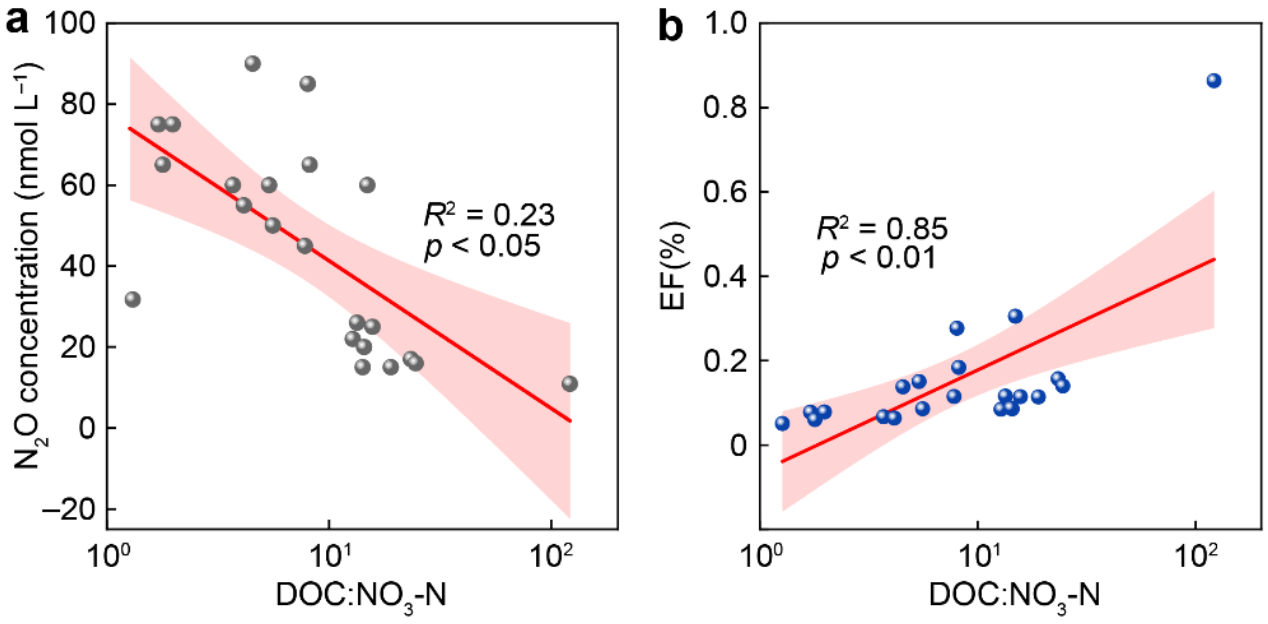


Fig. 7. Dissolved N_2O concentration and emission factor as functions of DOC/NO_3-N in estuaries globally. a, Relationship between dissolved N_2O concentration and DOC/NO_3-N. **b**, Relationship between EF_{5e} and DOC/NO_3-N. Red lines are the linear regression fits, with shaded areas showing the 95% confidence bands.

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Competing interests

The authors declare no competing interests.

Contributions

Y.D., S.W., S.X., and C.W. designed the study. Y.D. and C.W. wrote the first draft of the manuscript. Y.D., J.L., X.C., W.L., and C.Z. carried out the fieldwork. Y.D., J.L., X.C., F.F., W.L., C.Z, and C.L. performed lab analysis and data procurement. Y.D., X.C., Y.L., and C.L. conducted model simulations. Y.D., X.C., C.L., and C.W. prepared figures. All authors contributed to the interpretation and the preparation of the final manuscript.

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Supplementary Information

Disproportionately high nitrous oxide emissions from wastewater-influenced estuaries and the overestimation of IPCC emission factors

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Supplementary Figures

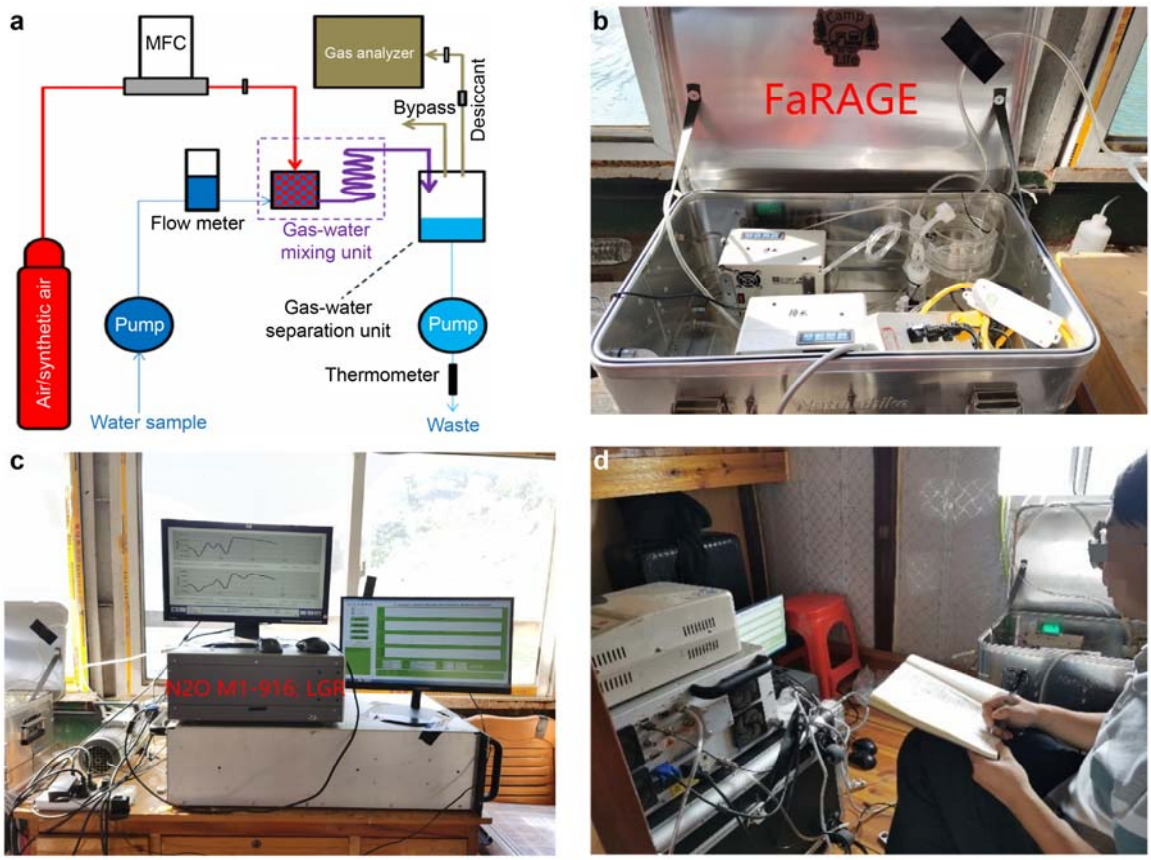

Fig. S1. High-resolution N_2O monitoring platform used in this study. a, Schematic the FaRAGE system[1]. **b**, Photo of the FaRAGE system used in the field campaign. **c**, The FaRAGE system was coupled with a portable gas analyzer (N_2O M1-916, LGR). **d**, A researcher monitoring the system.

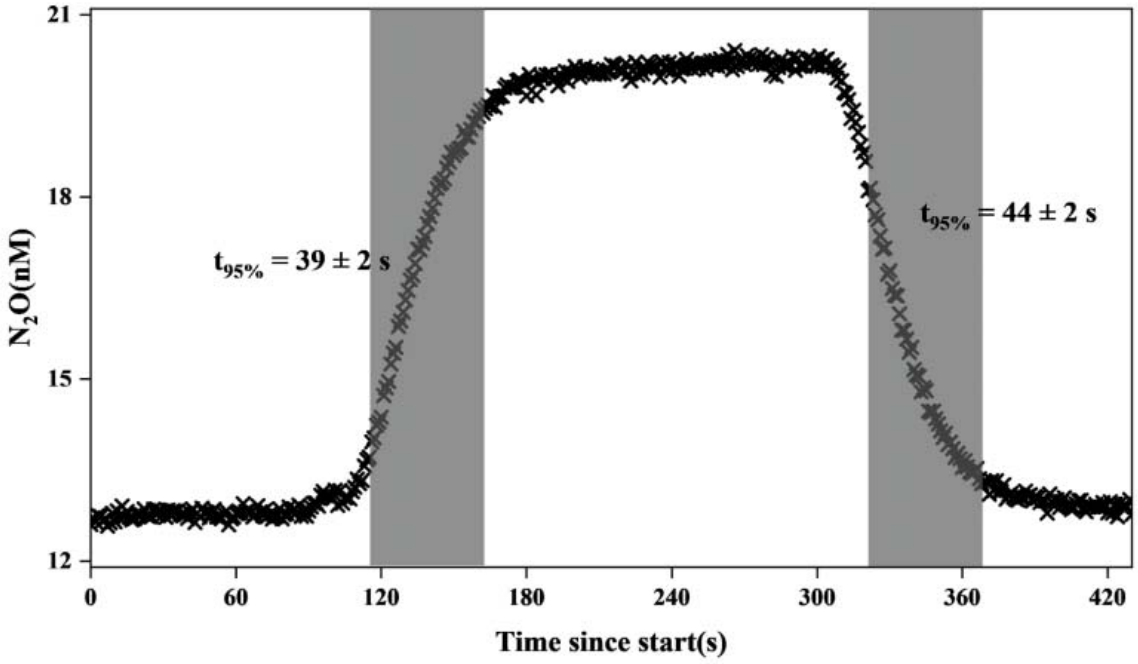

Fig. S2. Response process from the sample entering to the plateaus of measurement with a response time of 39±2s using the FaRAGE-based N_2O measurements.

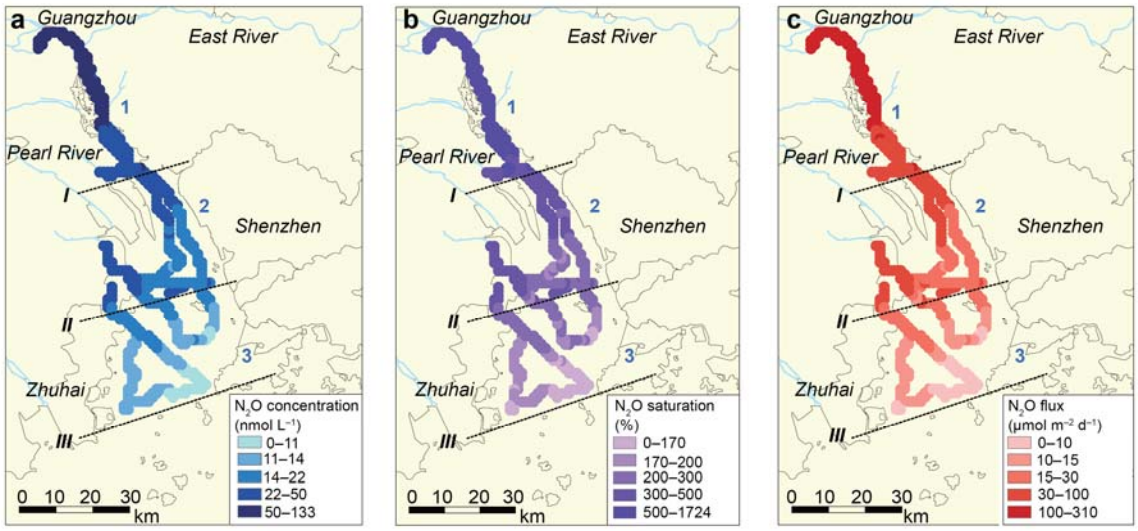

Fig. S3. Spatial distributions of N_2O concentration, saturation, and flux in PRE from the real-time, high-resolution monitoring. a, Dissolved N_2O concentration. **b**, N_2O saturation. **c**, N_2O flux.

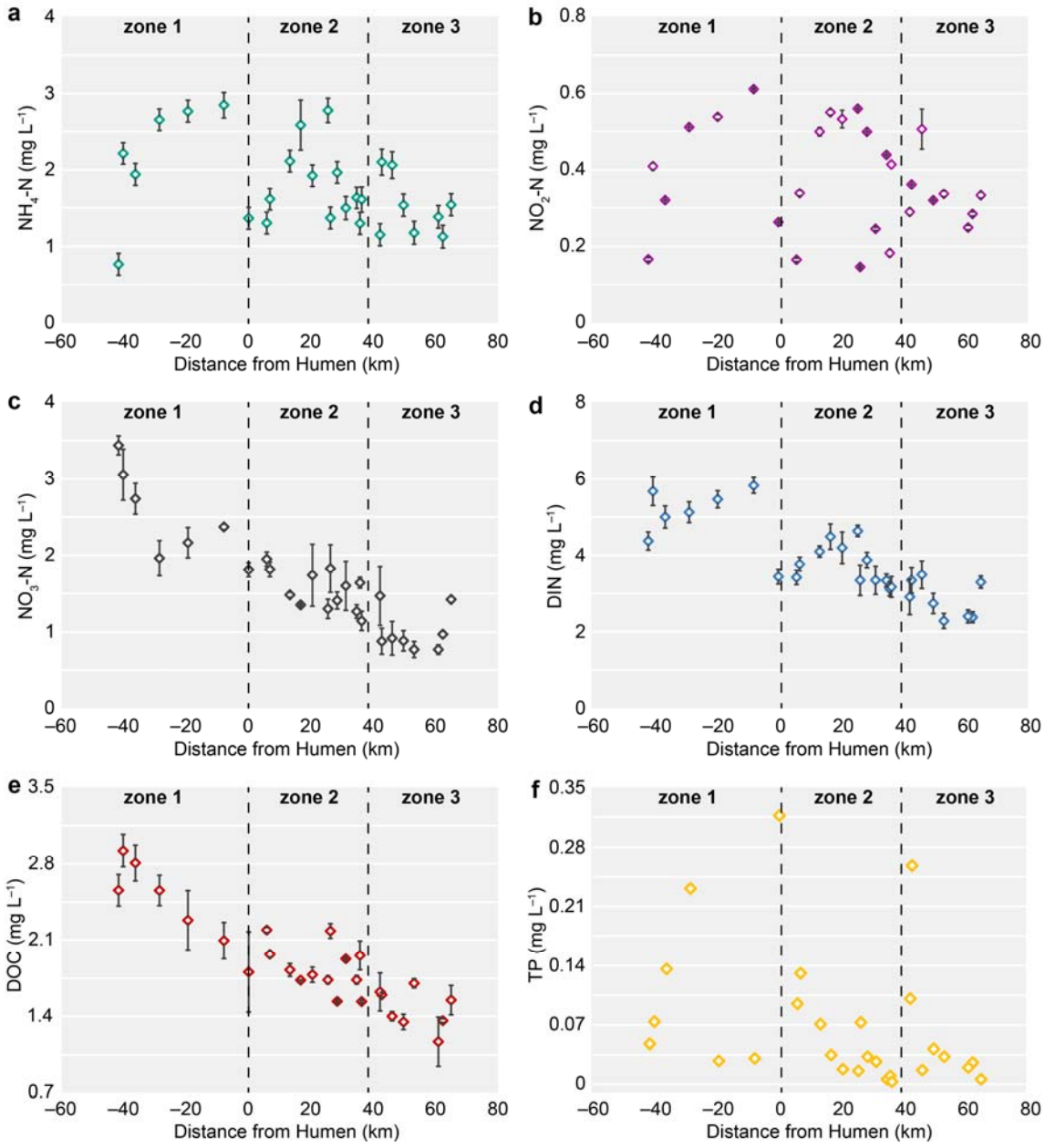

Fig. S4. Distribution of physicochemical parameters in PRE as a function of distance from Humen. a, NH_4-N. **b**, NO_2-N. **c**, NO_3-N. **d**, DIN. **e**, DOC. **f**, TP**.** The distance is positive downstream of Humen Outlet. Dashed lines in black delineate three zones of PRE: zone 1—upper Estuary (Humen upstream), zone 2—middle Estuary (Inner Lingdingyang), and zone 3—lower Estuary (Outer Lingdingyang).

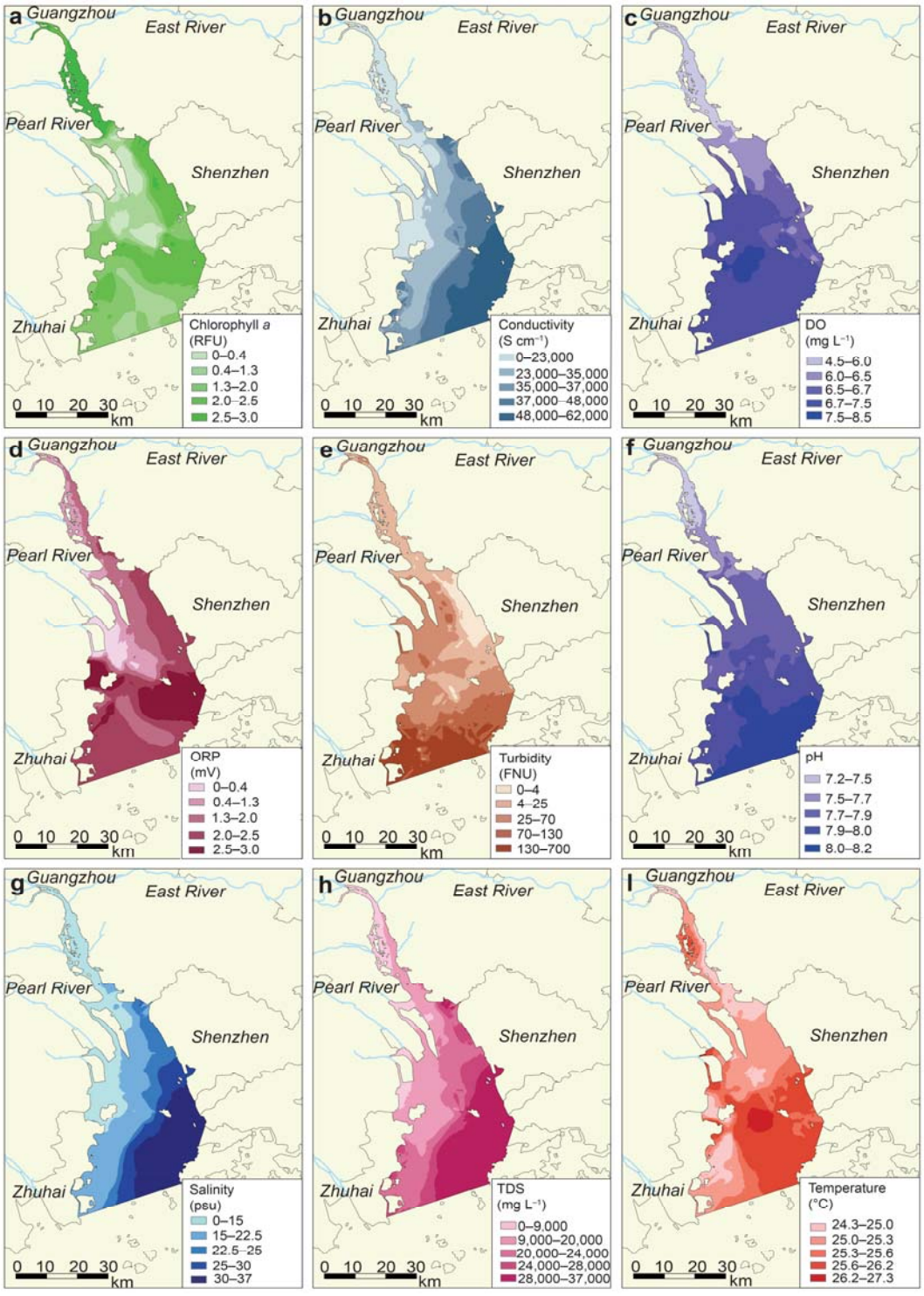

Fig. S5. Spatial distribution of physicochemical parameters. **a**, Chlorophyll a. **b**, Conductivity. **c**, DO. **d**, ORP. **e**, Turbidity, **f**, pH. **g**, Salinity. **h**, Total dissolved solids (TDS). **i**, Temperature.

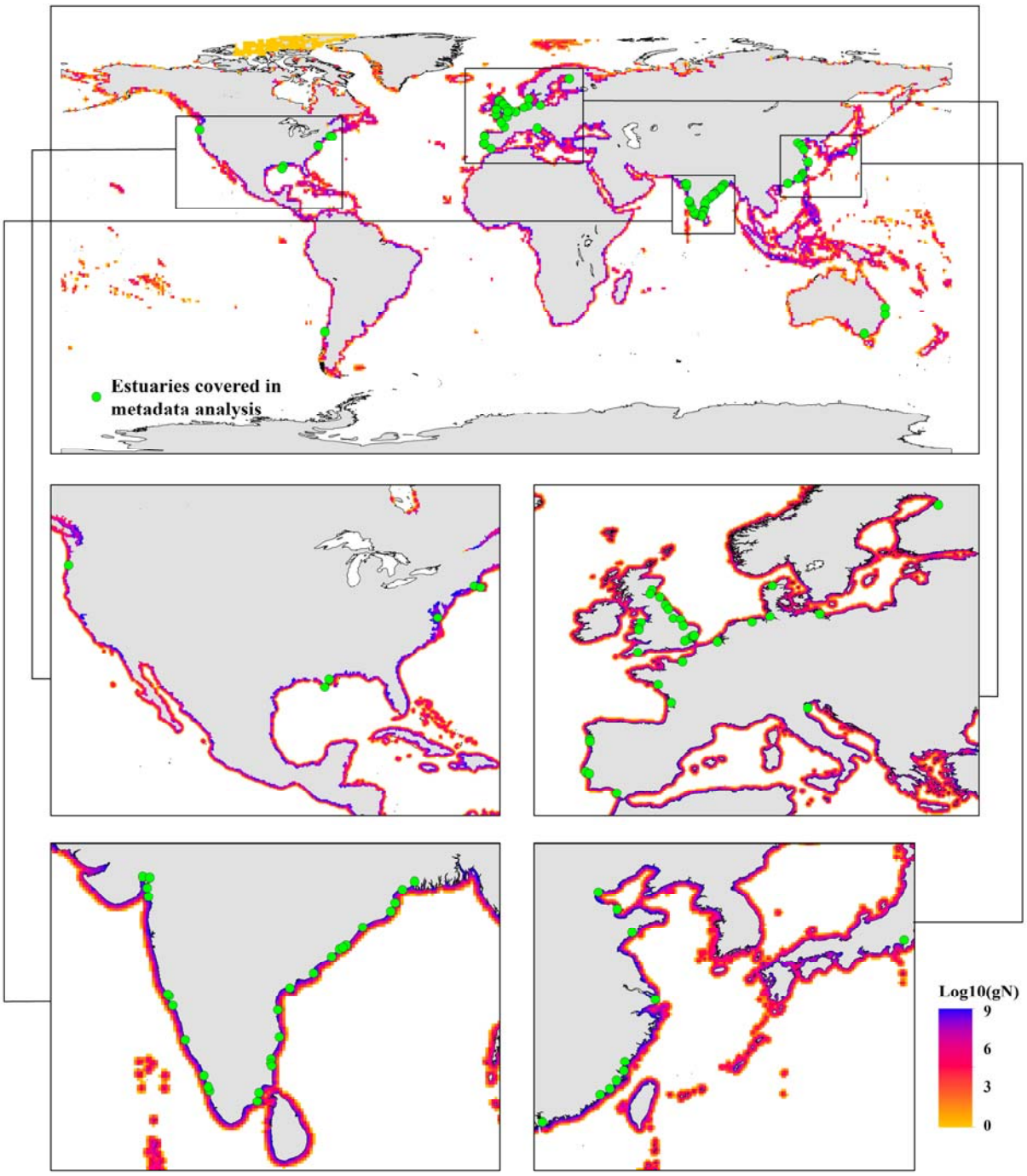

Fig. S6. The distribution of wastewater nitrogen input and estuarine location covered in the metadata analysis.

Supplementary Tables

Table S1. Summary of N_2O emissions in 83 estuaries globally synthesized from previous studies. Studies are classified into tropical (latitude range: 0–23.5°), subtropical (23.5–40°), and temperate (40–66.5°). Note: Lat. = Latitude; Sat. = Saturation; Ref. = Reference.

Lat. range	Estuary/Country	Dissolved N_2O	N_2O Flux	Sat.	EF_{5e}	NO_3-N	DOC: NO_3^--N	Ref.
		nmol L^{-1}	μmol m^{-2} d^{-2}	%	% N_2O-N /NO_3^--N	mg L^{-1}	mass:mass	
40–66.5°	Alsea, USA	11.79	5.52		0.275	0.12		[2,3]
	Bodden, Germany		3.29	202				[4,5]
	Childs River, USA	60.00	11.00					[6,7]
	Colne, UK	94.64				4.81		[3]
	Colne, UK			519				[8]
	Colne, UK				0.138			[9]
	Colne, UK	80.00		275				[10,11]
	Conwy, UK	18.60		114	0.197	0.26		[9]
	Deben, UK	32.60		187	0.046	1.99		[9]
	Elbe, Germany	18.2	48.10	201	0.033	1.54		[12]
	Elbe, Germany	18.6	48.10	201	0.02	2.66		[12]
	Elbe, Germany		33.60	202				[13,14]
	Ems, Germany		76.59	418				[13]
	Forth, UK	15.42	15.57	152				[13]
	Gironde, France	14.50	25.50	132				[5,14]
	Humber (from Humber Bridge to Spurn Head), UK	24.70		149	0.038	1.828		[9]
	Humber (from Trent falls to Humber Bridge), UK	32.10		187	0.023	3.92		[9]
	Humber, UK	67.87	76.59	396				[13]
	Jiaozhou Bay, China		14.20					[15]
	Lima, Portugal	36.79	4.85	170	0.644	0.16		[3,16]
	Limfjorden, Denmark		20.00					[17]
	Loire, France	14.15	14.20	168				[14,18]
	Minho, Portugal	33.57	4.05	109	0.495	0.19		[3,16]
	Sado, Portugal	8.73				0.011		[19]
	Sado, Portugal	5.57				0.017		[19]
	NarragansettBay, USA		1.44					[4,20]
	Waquoit Bay, USA	17.3				0.048		[21]
	Waquoit Bay, USA	17.4				0.026		[21]

	Waquoit Bay, USA	9.15				0.002		[21]
	Waquoit Bay, USA	8.40				0.001		[21]
	Chesapeake Bay, USA	10.16				0.231		[22]
	Chesapeake Bay, USA	9.17				0.066		[22]
	Chesapeake Bay, USA	10.66				0.039		[22]
	Chesapeake Bay, USA	5.42				0.118		[22]
	Chesapeake Bay, USA	7.12				0.003		[22]
	Chesapeake Bay, USA	7.42				0.003		[22]
	Orwell, UK	46.30		282	0.059	2.18		[9]
	Po River, Italy		21.60					[4,23]
	Scheldt, Netherlands			700				[11,24]
	Stour, UK	24.90		143	0.059	1.19		[9]
	Tagus Estuary, Portugal		2.88					[4,25]
	Tamar, UK	12.97	8.09	145				[13]
	Tamar, UK	15.36	9.84		0.030	1.41		[3,26]
	Tay, UK	18.20	2.49	104				[13]
	Tees, UK	33.47		383				[13]
	Temmesjoki, Finland	17.00	0.63	264	0.159	0.3		[4,27]
	Thames, UK	55.00	69.10	321				[14,18]
	Tianjin river, China	31.07	12.48		0.028	3.11		[3,28]
	Tokyo Bay, Japan	73.92	77.26	400				[29]
	Tweed, UK		43.20	100				[4,30]
	Tyne, UK	11.68	7.47	124				[13]
	Tyne, UK		31.20					[4,31]
	Wash, UK	19.43	3.74	106				[13]
	Werribee, Australia	68.00			0.181	1.05		[3,7]
	Yellow River, China				0.013	3.95		[3,32]
23.5-40°	Brisbane River, Australia		15.12					[11,33]
	Coffscreek, Australia	6.1	0.01	98	2.03	0.008	290.90	[34]
	Coffscreek, Australia	3.9	-4.81	65	3.90	0.003	1326.00	[34]
	Coffscreek, Australia	13.7	12.04	214	1.31	0.029	158.90	[34]
	Coffscreek, Australia	17	14.61	262	4.25	0.011	511.40	[34]
	Coffscreek, Australia	13.7	17.80	215	0.31	0.125	39.26	[34]
	Guadalete, Spain				0.196	1.736		[3,11]
	Gulf of Mexico, USA	6.00						[11,35]
	Gulf of Mexico, USA		2.40					[4,36]
	Jiaoxi River, China	17.00		130	0.157	0.30	23.40	[37]
	Jiaoxi River, China	16.00		135	0.140	0.32	24.60	[37]
	Jiaoxi River, China	15.00		125	0.088	0.48	14.20	[37]

	Jiaoxi River, China	15.00		120	0.114	0.37	19.00	[37]
	Jin River, China	90.00		600	0.138	1.83	4.54	[37]
	Jin River, China	85.00	8.73	600	0.277	0.86	8.03	[37]
	Jin River, China	65.00		420	0.184	0.99	8.19	[37]
	Jin River, China	60.00		400	0.305	0.55	14.90	[37]
	Jiulong River, China	75.00		550	0.078		1.71	[37]
	Jiulong River, China	75.00		550	0.078		1.98	[37]
	Jiulong River, China	65.00		450	0.060		1.79	[37]
	Jiulong River, China	60.00		475	0.151		5.38	[37]
	Jiulong River, China		32.20	380	1.12			[14,38]
	Jiulong River, China				0.031	1.04		[3,39]
	Min River, China	26.00		200	0.116	0.63	13.40	[37]
	Min River, China	25.00		200	0.114	0.61	15.70	[37]
	Min River, China	22.00		155	0.085	0.73	12.80	[37]
	Min River, China	20.00		150	0.086	0.65	14.40	[37]
	Mulan River, China	60.00		450	0.067	2.51	3.70	[37]
	Mulan River, China	55.00		410	0.064	2.42	4.14	[37]
	Mulan River, China	50.00		350	0.086	1.63	5.59	[37]
	Mulan River, China	45.00		325	0.115	1.09	7.79	[37]
	Pearl River, China	31.70	58.49	429	0.051	1.554	1.27	This study
	Pearl River, China	193.00		2404				[40]
	Pearl River, China	126.43	37.00	450		1.51		[3,14]
	Pearl River, China	37.50						[41]
	Trent, UK	32.10		187	0.023	3.92		[9]
	Tubul_Raqui, Chile			67				[42]
	Yangtze River, China	18.93		212	0.010			[43]
	Yangtze River, China	15.90	52.94	258				[44]
	Yangtze River, China		13.30	137				[45]
0-23.5°	Adyar, India	19			0.35	0.15		[46]
	Adyar, India	7			0.16	0.13		[46]
	Adyar, India	10			0.11	0.25		[46]
	Adyar, India	44			1.76	0.07		[46]
	Adyar, India	12			0.11	0.31		[46]
	Adyar, India	34			0.15	0.63		[46]
	Adyar, India	30			0.67	0.13		[46]
	Ambalayar, India	8.00	0.18	125				[47]
	Bay of Bengal, India	6.32	12.42	106				[48]
	Bharatakulza, India			169				[47]

	Cauvery, India			221				[47]
	Chalakudi, India	11.00		180				[47]
	Godavari, India			162				[47]
	Haldia, India			148				[47]
	Kali, India	16.60	0.26	240				[47]
	KochiBlackwater, India	24.80	3.42	385				[47]
	Krishna, India			113				[47]
	Mahisagar, India	47.10		567				[47]
	Mandovi, India	9.30	0.43	141				[47]
	Nagavali, India	19.80		249				[47]
	Narmada, India			333				[47]
	Netravathi, India	14.90	1.76	236				[47]
	penna, India			274				[47]
	ponnayaar, India			5902				[47]
	Rushikulya, India	4.90		75				[47]
	Sabarmathi, India			214				[47]
	Tapti, India	42.50	14.21	556				[47]
	Vaigai, India	22.10	3.07	327				[47]
	Vamsadhara, India	8.40		122				[47]
	Vellar, India			125				[47]
	Zuari, India	12.50	12.50	195				[47]

Table S2. Statistics of wastewater nitrogen discharge in 83 estuaries globally derived from Tuholske et al.'s dataset[49].

Estuary/Country	Wastewater nitrogen discharge (Mg N/km^2)		
	Maximum	Mean	Standard deviation
Tianjing, China	386.39	23.04	55.65
Huanghe River, China	632.04	56.01	128.5
Jiaozhou Bay, China	23.28	3.81	5.96
Yangtze River, China	294.44	2.66	16.73
Jiaoxi River, China	3.58	0.62	0.85
Mulan River, China	15.96	1.44	3.02
Jin River, China	41.32	3.96	8.04
jiulong River, China	33.35	3.66	8.22
pearl River, China	727.92	12.7	52.13
Min River, China	86.86	5.29	14.6
Brisbane River, Australia	1.01	0.06	0.16
Coffscreek, Australia	0.04	0	0.01
Werribee, Australia	1.9	0.12	0.28
Limfjorden, Denmark	0.04	0.01	0.01
Tay, UK	2.11	0.3	0.52
Forth, UK	5.72	0.73	1.26
Tweed, UK	0.99	0.07	0.18
Tyne, UK	9.65	1.46	2.45
Tees, UK	1.29	0.22	0.37
Wash, UK	23.04	1.6	3.83
Tamar, UK	3.89	0.41	0.83
Conwy, UK	0.55	0.1	0.17
Mawddach, UK	0.07	0.01	0.01
Dovey, UK	0.31	0.04	0.06
Seine, France	0.34	0.06	0.1
Loire, France	0.26	0.05	0.07
Gironde, France	6.3	0.32	0.98
Scheldt, Netherlands	3.61	0.23	0.62
Ems, Germany	11.72	0.76	2.21
Elbe, Germany	166.71	13.27	31.61
Bodden, Germany	0.66	0.05	0.1

Temmesjoki, Finland	2.59	0.13	0.37
Po River, Italy	150.82	13.24	30.43
Minho, Portugal	6.57	0.72	1.42
Lima, Portugal	3.05	0.43	0.72
Tagus Estuary, Portugal	11.09	1.71	3.05
Sado estuary, Portugal	0.93	0.12	0.21
Guadalete, Spain	4.85	0.33	0.79
Childs River, USA	0.31	0.03	0.07
NarragansettBay, USA	10.02	0.82	2.02
Sub_Gulf Coast, USA	0.01	0	0
Gulf of Mexico, USA	0.01	0	0
Alsea, USA	0.04	0.01	0.01
Chesapeake Bay,USA	46.40	1.62	4.38
Tubul_Raqui, Chile	6.71	0.26	0.77
Humber, UK	77.62	5.71	13.97
Thames, UK	9.87	1.21	2.09
Colne, UK	3.66	0.81	1.14
Orwell, UK	0.88	0.18	0.2
Deben, UK	1.08	0.22	0.33
Trent, UK	77.62	5.71	13.97
Ouse, UK	77.62	5.71	13.97
Stour, UK	0.88	0.18	0.2
Tokyo Bay, Japan	22.29	2.24	4.31
Haldia, India	104.66	8.4	19.61
Subarnalekha, India	24.74	2.56	5.3
Baitarani, India	39.45	2.48	6.88
Mahanadi, India	102.56	6	17.25
Rushikulya, India	8.15	0.79	1.7
Vamsadhara, India	6.42	0.85	1.55
Nagavali, India	7.66	0.95	1.77
Bay of Bengal, India	1.96	0.15	0.26
Visakhapatnam, India	3.02	0.38	0.68
Godavari, India	207.89	17.75	41.63
Krishna, India	240.79	15.35	42.9
penna, India	34.04	3.15	7.04
Adyar, India	14.97	2.08	3.56

ponnayaar, India	33.84	3.06	6.62
Vellar, India	50.64	4.62	8.78
Cauvery, India	96.35	11.8	23.04
Ambalayar, India	2.79	0.61	0.85
Vaigai, India	11.4	2.02	3.5
KochiBlackwater, India	16.13	1.6	3.54
Chalakudi, India	10.31	1.19	2.06
Bharatakulza, India	18	1.86	3.75
Netravathi, India	6.36	0.75	1.4
Kali, India	1.67	0.2	0.38
Zuari, India	2.89	0.31	0.64
Mandovi, India	2.98	0.55	0.89
Tapti, India	81.87	7.61	16.56
Narmada, India	65.88	10.32	16.44
Mahisagar, India	39.57	23.44	7.49
Sabarmathi, India	30.7	22.35	5.76

Table S3. Primer pairs used in qPCR assays and the corresponding amplification conditions.

Gene	Primer	Sequence (5'-3')	Thermal profile	Reaction mixture	Amplification efficiency (%)	Ref.
AOA *amo*A	arch-amoA-23F	ATGGTCTGGCTWAGACG	95°C for 3 min, 40 cycles of 5 s at 95°C, 30 s at 58°C, and 1 min at 72°C	10 µL 2X ChamQ SYBR Color qPCR Master Mix, 0.8 µL of each primer (5 µM), 0.4 µL 50 X ROX Reference Dye 1, and 2 µL Template (DNA)	89	[50]
	arch-amoA-616R	GCCATCCATCTGTATGTCCA				
AOB *amo*A	CTO189f	GGAGRAAAGCAGGGGATCG			105	[51]
	CTO654r	CTAGCYTTGTAGTTTCAAAC GC				
nirS	cd3aF	GTSAACGTSAAGGARACSGG			104	[52]
	R3cd	GASTTCGGRTGSGTCTTGA				
nirK	FlaCu	ATCATGGTSCTGCCGCG			101	[53]
	R3cu	GCCTCGATCAGRTTGTGGTT				
narG	narG-f	TCGCCSATYCCGGCSATGTC			99	[54]
	narG-r	GAGTTGTACCAGTCRGCSGAYTCSG				
nosZ	nosZF	CGYTGTTCMTCGACAGCCAG			96	[55]
	nosZR	CATGTGCAGNGCRTGGCAGAA				

Table S4. Goodness of fit for statistics of linear, efficiency loss and Michaelis-Menten model in global, Asian, European, American and Oceania estuaries.

		RSE	Adjusted R^2	p-value	RMSE	AIC
Global	Michaelis-Menten	0.304	0.254	2.547e-41	0.298	32.20
	Linear	0.299	0.274	6.480e-06	0.294	30.50
	Efficiency loss	**0.250**	**0.489**	**1.078e-10**	**0.246**	**8.28**
Asian	**Michaelis-Menten**	**0.269**	**0.281**	**3.367e-19**	**0.258**	**9.43**
	Linear	0.306	0.072	0.100	0.294	16.10
	Efficiency loss	0.277	0.238	0.007	0.266	11.00
European	Michaelis-Menten	0.232	0.423	5.397e-13	0.218	2.46
	Linear	0.256	0.299	0.014	0.240	5.77
	Efficiency loss	**0.233**	**0.419**	**0.003**	**0.219**	**2.57**
American and Oceania	Michaelis-Menten	0.248	0.149	1.216e-10	0.233	4.65
	Linear	**0.1898**	**0.504**	**0.001**	**0.178**	**-4.50**
	Efficiency loss	0.2168	0.351	0.007	0.2038	0.042

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RESPONSES TO REFEREES

We would like to express our sincere gratitude to the reviewers for their valuable suggestions, which have greatly contributed to the improvement of this manuscript.

Based on the comments received, we have thoroughly revised the manuscript. All changes we made in this round of revision have been highlighted in yellow in the main text, and a clean version is also provided. Please find below our point-by-point responses, with the reviewers' comments highlighted in red.

Reviewer 1:

The revised manuscript has been streamlined and improved. My major comments have been addressed. However, there are still a number of minor grammatical issues throughout (e.g. missing commas), and these should be addressed prior to final publication.

Response: Thanks for your valuable comment. We have thoroughly checked the entire manuscript to correct grammatical issues. All changes have been highlighted in the manuscript.

Reviewer 2:

I revised an earlier version of this manuscript (with the title: Significant nitrous oxide emissions but overestimated IPCC emission factors in wastewater-influenced estuaries) and despite some concerns I indicated this to be solid work that is suitable for the journal. The issues raised by me during the first round of review were mostly related with missing in-depth analysis of the physical and biogeochemical setting in which the measurements were conducted and how this forcing actually impacts N2O emissions and emission factors.

After revising the authors' response letter and the revised manuscript, it is my opinion that all those concerns have been adequately addressed by including additional data, analysis and discussion. At this point, I only have minor comments which I included in the attached version of the main manuscript and supplementary material. While most of these comments are related to formatting issues, I would like to emphasize that the comments to the methods section need to be addressed before publication because the current version of the manuscript lacks of clarity in essential aspects of the analytical system used. I don't expect this to affect the study's conclusions, but it is important to ensure that this research is reproducible.

Response: Thanks for your positive comment. We have revised the manuscript carefully based on the comments in the attachment. The detailed response are listed as follows.

Line 28: "through introducing" – influence on what? Emissions? Also, I would replace "through" with "by"

Response: We have changed this to "Wastewater discharge substantially impacts emissions by introducing"…

Lines 50-51: Recent work highlights the important role of coastal vegetation in estuarine systems as a driver for N2O emissions. The authors might want to refer to and cite this work:
Rosentreter, J.A., Laruelle, G.G., Bange, H.W. et al. Coastal vegetation and estuaries are collectively a greenhouse gas sink. Nat. Clim. Chang. 13, 579–587 (2023). https://doi.org/10.1038/s41558-023-01682-9
Response: Thanks for pointing this out. We have added "coastal vegetation" and cited this paper.

Line 52: "is important" – "is important"
Response: We have changed this part to "are critical contributors to"

Line 89: Change "samples" to "measurements".
Response: Done.

Line 129: "loading" - Perhaps "load" is more appropriate here.
Response: We have changed it to "load".

Line 189: "emission" – "emissions"
Response: Done.

Line 276: "Maavara et al. developed…" - Add year.
Response: We changed this sentence to "A mechanistic modeling approach for predicting N2O emissions was developed previously[12], …"

Line 282: "Albeit with these advancements" – remove "with"
Response: We changed to "Despite these advancements".

Line 363: Change "measurement" to "measurements".
Response: Done.

Lines 364-365: "water samples are continuously pumped" - I would not talk about "samples" on a system for continuous measurements.

Response: We have changed to "water is continuously …"

Line 371: "was 39±2 s (Fig. S2)." - Yet figure S2 shows two different values for the different directions of change between low and high concentrations. The response time should be then a mean value of all the experiments. Please revise.

Response: Thanks for the suggestion. We have changed this to "41±2 s".

Line 373: Why does the system needs a carrier gas? This needs to be explained. Also, please state what was the mole fraction of N2O in the carrier gas.

Response: Thanks for the question. Carrier gas such as N_2 is widely used in greenhouse gas monitoring to mobilize the sample by pushing it through the system. We actually used pure N_2 gas as the carrier gas, which has been specified in the revision: "carrier gas (N2, 99.9%)."

Line 376: "Please include information on how the gas analyzer was calibrated or if the data was post-corrected."

Response: We did not perform post-correction for data, and the calibration of gas analyzer is routinely performed at the factory, which is outside the scope of our field measurements.

Equation 7: Equation 5 already includes a normalized Sc. Moreover, since 600 is used for freshwater and 660 for seawater, the authors should clearly state a criteria followed to apply this term differentially to the different zones along the salinity gradient.

Response: We have revised the method of gas transfer velocity calculation. The Sc is normalized with 600 in freshwater and 660 in seawater. And freshwater condition is used for the upstream of Humen (upper estuary; zone 1), seawater condition is used for Lingdingyang (middle and lower estuary; zone 2 and 3). The details was highlighted in yellow in the manuscript.

Line 475: "concertation" – "concentration"

Response: Done.

Lines 491-494: This sentence is incomplete. Please revise.

Response: We have revised this sentence to "Yellow circles indicate sampling sites where

biogeochemical parameters (NH_4-N, NO_2-N, NO_3-N, dissolved inorganic nitrogen (DIN), dissolved organic carbon (DOC), and total phosphorus (TP)) as well as ammonia-oxidizing and denitrifying microbial genes with qPCR (AOA *amo*A, AOB *amo*A, *nirS*, *nirK*, *nosZ*, and *narG*) were analyzed."

Line 501: Change "fluxes" to "air-sea fluxes"

Response: We have changed this to "air–water fluxes".

Line 506: "submarine groundwater" - I appreciate the authors made an effort ion including the potential effects of submarine groundwater discharge. However, in this version of the manuscript is not clear how these data were acquired and/or measurements were carried out. I therefore invite the authors to include a few lines on this.

Response: The data of submarine ground water discharge was referred from this work: "*Liu, J., Du, J., Wu, Y. & Liu, S. Nutrient input through submarine groundwater discharge in two major Chinese estuaries: the Pearl River Estuary and the Changjiang River Estuary. Estuar. Coast. Shelf Sci. 203, 17 – 28 (2018).*"

We also added related description in the body text:"By applying the ^{224}Ra mass balance model, submarine groundwater discharges in the PRE are estimated to contribute water flows of (4.5–10) $\times 10^8$ m^3 d^{-1} and (1.2–2.7) $\times 10^8$ m^3 d^{-1} in the dry season and wet season, respectively[40]."

Line 26 in Supplementary Information: I think the label on the figure is misplaced as it is showing a Picarro analyzer and not an LGR analyzer. Also the authors indicate a portable analyzer is used, but in the picture one sees a rack-mount version instead. This is relevant, as the specification of the portable analyzers (e.g. accuracy) are different to those from rack-mount versions. Please clarify.

Response: The figure is revised and shows a LGR analyzer (the white device at the bottom) in Supplementary Figure 1. In fact, the Chinese agent sold this device as a portable analyzer, but it is indeed a rack-mount version, which is a translation issue with imported equipment. We have corrected related descriptions in the whole manuscript.

Line 32 in Supplementary Information: There are two distinc values of tau. The tau should be then an average of the values obtained in both phases.

Response: The average value of "41±2s" obtained in both phases was used to indicate the response time in Supplementary Figure 2.
