Cover note to the revised submission

Dear Editor,

We thank the two referees and the Associate Editor for the time and care given to our manuscript. We summarise here the revision process, so that the structure of this resubmission is clear.

In the first round, both referees provided supportive and constructive comments. We addressed each of them point by point and revised the manuscript accordingly, in particular by clarifying the role of nitrite as the central dissolved intermediate of the nitrogen cycle, reducing repetition between sections, adding a dedicated treatment of the analytical limitations of nitrite isotope measurements, and updating the manuscript with the most recent literature.

The Associate Editor then returned a synthesis of the two reports, noting that both referees converged on two points that deserved more thorough and consistent treatment: the framing of nitrite as the “only stable” inorganic nitrogen species, and the challenge that nitrite is neither stable nor always quantifiable in natural systems. In response, we have carried out a further round of revision. We have grounded the centrality of nitrite in its structural position at the +III oxidation state rather than in chemical stability, and we have applied this framing consistently across the abstract, the introduction, and the conclusions. We have also strengthened the treatment of the analytical limitation at every level of the text, and incorporated the Associate Editor’s specific suggestions.

Because this second round of revision modifies the wording on which several of our earlier replies to the referees were based, we have kept those earlier replies intact and added, beneath each, an update describing the further change, so that the responses remain consistent with the present version of the manuscript while preserving the record of the first round. The updated responses, together with our response to the Associate Editor, follow below.

We hope that the revised manuscript now meets the standards for publication in Biogeosciences.

Sincerely,

Mathieu Sebilo and Rosanna Margalef-Marti

Response to the Associate Editor

Dear Dr Janssen,

We thank you for handling our manuscript and for your synthesis of the two referee reports. We are grateful that both referees and you find the framework valuable, and we have used your guidance to address the two recurring points more thoroughly and consistently across the manuscript, including the abstract and conclusions. We respond below to each of your points, and we provide updated point-by-point responses to Referee 1 and Referee 2 in the accompanying sections.

On the framing of nitrite as the "only stable" inorganic nitrogen species, where both referees suggested alternate framings. We have reconsidered this wording throughout the manuscript and grounded the claim in structure rather than stability. Nitrite occupies the +III oxidation state, the obligatory redox transit between the reductive and oxidative domains of the network; this is a topological property fixed by redox stoichiometry, not a statement about chemical persistence. We have revised the abstract (formerly "only inorganic nitrogen species"), the introduction (formerly "central stable dissolved intermediate", now "principal dissolved inorganic intermediate"), and the relevant passages so that the framing now rests consistently on this structural argument. Where a comparative descriptor is retained, we use "principal" rather than "only". This reframing also resolves the internal tension you identified: our acknowledgement that nitrite is chemically labile is no longer in contradiction with its central role, since centrality is now established on structural grounds. This directly addresses Referee 1's question on whether nitrite is truly the "only" species linking both regimes (P3 L63-76) and Referee 2's parallel caution on the same wording; both are now resolved on the same structural basis.

On the challenge that nitrite is not stable and not always quantifiable, which both referees highlighted. We agree this is fundamental, and we have addressed it at every level of the text. We have reframed the limitation conceptually: the low and frequently undetectable concentration of nitrite is the direct kinetic expression of the very centrality we argue for. Under quasi-steady-state conditions, an intermediate whose consumption rate greatly exceeds its rate of supply sustains high gross flux at near-zero standing concentration. We have made this argument explicit in the abstract, incorporated it into the Kinetic control section, where we now distinguish the regime in which consumption is limited only by supply so that concentration itself — not merely its rate of change — approaches zero, and reflected it in the legend of Figure 2. We have developed the analytical limitations in the abstract and in a dedicated passage of the Isotopic constraints section, covering the chemical instability of nitrite, its low environmental concentrations, preservation requirements, and current detection limits, including a recent high-sensitivity method reaching 0.02 µmol L^{-1} (Jiang et al., 2026). This consolidates our responses to Referee 1's request for a dedicated limitations passage and to Referee 2's detailed comments on detection limits and transition-zone constraints (P10 L266-287, P13 L362-366).

On overcoming the limitation. Following your invitation, we have expanded the discussion of complementary approaches while drawing a distinction we believe strengthens the framework. Tracer additions and molecular or transcriptomic measurements characterise the *potential* for nitrite turnover and necessarily perturb the system they interrogate. Natural-abundance isotope

analysis of an in situ sample is alone non-perturbative: it records the actual *expression* of turnover under real conditions. The complementary methods therefore address a different question and cannot substitute for the in situ signal. We also clarify that the principal analytical challenge is ultimately not detection of the nitrite pool but deconvolution of a composite in situ signature integrating concurrent production and consumption with their respective fractionation factors.

We respond to your specific minor suggestions below, and to the detailed comments of both referees in the accompanying point-by-point sections.

Responses to the Associate Editor's specific suggestions

Line 20 – I suggest "...ammonium – while pathway…"

Adopted (P2 L20). The sentence now reads: *"Concentration-based assessments emphasize the dominant pools — nitrate and ammonium — while pathway divergence is determined at the stage of nitrite turnover, independently of pool size."*

Lines 59-60 – I suggest "Despite NO also participating…"

Adopted (P3 L63-76), with a reformulation that grounds the distinction in structure rather than stability. The passage now reads: *"The central role of nitrite follows from its position in the reaction network. It occupies the +III oxidation state, midway between ammonium and nitrate., and every transformation linking oxidative and reductive branches passes through it. (Burgin & Hamilton, 2007; Lam & Kuypers, 2011; Kraft et al., 2014). Nitric oxide (NO) also participates in both branches. Its half-life of seconds in aqueous solution keeps its concentration below measurable levels under environmental conditions, which leaves nitrite as the single dissolved intermediate accessible to direct observation. No other inorganic nitrogen compound combines this dual role of convergence and divergence."*

Lines 112-114, 151-153, Figs 1 & 3: Mn(III) also reacts with NO2- abiotically, forming NO3- , and with constrained isotope effects. See Karolewski et al., 2021 and Luther et al., 2021.

We thank you for this addition. We have incorporated the abiotic oxidation of nitrite to nitrate by ligand-bound Mn(III) at two points in the text.

In the reaction-network description (P5 L143-144), the text now reads: *"Abiotic reactions extend this network in both redox directions: reduction of nitrite by ferrous iron generates NO and N_2O (Jones et al., 2015; Grabb et al., 2017; Robinson et al., 2021), while oxidation of nitrite to nitrate by ligand-bound Mn(III) proceeds abiotically under both oxic and anoxic conditions (Karolewski et al., 2021; Luther et al., 2021)."*

In the isotopic section (P9 L237-241), we have added: *"Abiotic oxidation of nitrite to nitrate by ligand-bound Mn(III) imparts a large inverse nitrogen isotope effect (≈ −20‰) closely resembling that of biological nitrite oxidation, with the additional oxygen atom derived from water (Karolewski et al., 2021). This convergence of biotic and abiotic fractionation factors reinforces the value of interpreting isotopic signatures within environmental and redox context."*

We have chosen to retain Figures 1 and 3 unchanged: both already represent abiotic chemodenitrification, and adding a further abiotic arrow would crowd schematics whose legibility depends on a limited number of pathways. The Mn(III) oxidation pathway is now fully described and referenced in the text.

> *P13 L350 "Concentration reflects net balance between production and consumption": Changes in concentration reflect the net balance. The concentration itself at a given moment is not dependent on net balance, and high or low instantaneous NO2- concentrations could theoretically exist at the same or similar net balance state.*

This is correct and we have amended the statement. The sentence now reads: *"Changes in concentration reflect the net balance between production and consumption, whereas $\delta^{15}N$ records the imprint of concurrent production and consumption even when pool size remains constant."* We have used this correction to reinforce a central point: because the instantaneous concentration is not uniquely determined by the net balance, two systems in similar net states may hold very different standing nitrite concentrations, while the isotopic composition continues to evolve in response to gross turnover even where the concentration itself is invariant or vanishingly small.

> *Lines 308-309: This could use a reference.*

A reference has been added (P13, L375). The closing passage on increasing redox variability now cites Callbeck et al. (2026), whose study of seasonal lake denitrification under climate warming directly documents the climate sensitivity of nitrogen loss invoked here. We note that this reference was published on 22 May 2026, after our initial submission on 15 April 2026, which is why it was absent from the original manuscript; it is incorporated here in response both to your suggestion and to a related comment from Reviewer 2.

Response to Referee 1

We sincerely thank the reviewer for their positive assessment of our manuscript and for highlighting the relevance of a nitrite-centered perspective in nitrogen cycling. We greatly appreciate the constructive and detailed feedback provided.

We agree that the manuscript required updating and better integration of recent advances in the field. In particular, we acknowledge the omission of several important and recent studies on nitrite dynamics, isotope characteristics, and modelling approaches. We have carefully incorporated the suggested references and revised the manuscript accordingly to reflect the latest developments and perspectives.

Detailed comments

> *P3, L55-56 "Nitrite is the only inorganic nitrogen species produced by both oxidative and reductive processes" – not really, what about N2O?! it can be produced due to nitrate reduction and as well from ammonia and hydroxylamine oxidation – this nitrification pathway is missing in the Figure 1!*

We thank the reviewer for this important clarification. Our intention was to emphasize the central role of nitrite as the key intermediate linking oxidative and reductive nitrogen transformations, rather than to imply that no other nitrogen species can be produced under both regimes. To avoid this ambiguity, we have revised the sentence to: "Nitrite is the central dissolved intermediate linking oxidative and reductive nitrogen transformations and the obligatory precursor to downstream nitrate, ammonium and gaseous nitrogen products." We have also explained in detail the role of hydroxylamine in the caption of Figure 1.

<u>Update following the Associate Editor's comments</u>. In the second round of revision, and consistently with Reviewer 2's parallel comment, we have grounded nitrite's centrality in structure: nitrite occupies the +III oxidation state, the redox transit between the reductive and oxidative branches of the network. The sentence now reads: "Nitrite is the principal dissolved inorganic intermediate linking the oxidative and reductive branches of the nitrogen cycle and the obligatory precursor to downstream nitrate, ammonium and gaseous nitrogen products." P2 L22-23.

> *P3 L62 you should also add newer N-cycle reviews here, eg. Denk et al., 2017, Deb et al, 2024.*

We have added these recent reviews to the revised manuscript in the section introducing major nitrogen transformation pathways and recent perspectives on nitrogen cycling.

> *P4 L73 also some more recent technical developments of nitrite isotope measurements should be mentioned: Deb and Lewicka-Szczebak, 2025 (doi.org/10.3389/fenvs.2025.1536882), Hu et al., 2026 (doi.org/10.1039/D5JA00363F)*

The reviewer is fully right and these interested references have been added.

> *P5 L122 – this was also observed in the recent case study (Deb et al., 2025)*

We have now included this reference in the revised manuscript as an additional example supporting the interpretation of simultaneous nitrite oxidation and reduction processes in environmental systems.

P5 L 136 – the nitrifier denitrification pathway that you describe here is also omitted in your Fig.1

We have explained in detail the role of hydroxylamine in the caption of Figure 1

P6 Fig 2 – I don't understand the bottom left panel illustrating delta 15N for dominating production, what do the 2 arrows mean? Two following graphs are clear, present the expected possible trends in isotope changes, but the first graph do not show change in time, just as one-point?

When nitrite production is higher than its consumption, the isotopic composition of N-nitrite will be initially depleted in 15N compared to the substrate and then it will evolve towards the initial isotopic 15N of the substrate, if it is limiting. We totally agree that this is not clear in the original version of Figure 2 and we have modified it accordingly in the revised version of the manuscript. We have also distinguished the isotopic effect of consumption during oxidative and reductive pathways. P7 L198 – the sentence beginning with "Direct determination…" is a totally different topic, you said about analytical techniques before and here the paragraph was about N2O. Also, a citation for N2O reduction overprinting its isotopic signal should be added here.

We agree that the sentence discussing direct nitrite isotope determination interrupted the logical flow of the paragraph focused on N_2O isotopic signatures. This sentence has therefore been removed. We have revised the paragraph structure to improve the transition between analytical considerations and N_2O isotope systematics. We also added references discussing isotopic overprinting associated with partial N_2O reduction.

P9 Fig.3 – really great figure with perfect presentation of nitrite central role

We thank the reviewer for this positive assessment of Figure 3 and are pleased that the figure effectively conveys the central role of nitrite turnover within the nitrogen cycle framework developed in this perspective.

P10 L262 The oxygen exchange with water is also an important tracer because allows us to estimate the nitrite residence time and rates of biological NO2 turnover relative to abiotic exchange (Lewicka-Szczebak et al., 2021, Buchwald and Casciotti, 2013)

We thank the reviewer for this important point. We agree that oxygen isotope exchange with water not only constrains interpretation of δ18O signatures, but may also provide valuable information on nitrite residence times and the relative rates of biological turnover versus abiotic exchange. We have revised the text accordingly.

P10 L279 I am missing the chapter on limitation of this approach. Most difficult one is the chemical instability of nitrite and its very low concentrations. If improperly stored and not analysed immediately it is quickly partially oxidised to nitrate and the isotope signal can be totally changed. Therefore, the analysis should be performed immediately, and the samples

must be carefully conserved, best with high pH conditions. Because of typically low nitrite concentrations, especially in terrestrial environments, the development of new analytical methods for lowering the detection limit for its isotope analysis is critical (Deb and Lewicka-Szczebak, 2025). I think these are important points because these analytical challenges are most probably the main reasons why nitrite isotope analyses in terrestrial systems are still very rare.

We agree that analytical limitations are a major factor restricting the broader application of nitrite isotope approaches in environmental systems. We have now added a paragraph discussing nitrite instability, rapid post-sampling transformation, low environmental concentrations, and the need for improved preservation and high-sensitivity analytical methods. This issue was also raised by reviewer 2.

Paragraph added in the Isotopes section: “Despite their strong mechanistic potential, nitrite isotope measurements are only feasible when sufficient nitrite accumulates for analysis and remain analytically challenging. Nitrite is chemically unstable and may undergo rapid oxidation or reduction during sampling and storage, potentially altering its isotopic composition before analysis. Reliable preservation therefore requires rapid processing and carefully controlled storage conditions, often under alkaline conditions that minimize transformation rates. In many terrestrial and aquatic systems, low nitrite concentrations restrict isotope analyses to zones where nitrite transiently accumulates. This emphasizes the need for continued development of high-sensitivity analytical approaches (Lewicka-Szczebak et al., 2021; Deb and Lewicka-Szczebak, 2025). For instance, a recent study developed a method for freshwater samples by coupling anion-exchange resin preconcentration with the azide reduction method for nitrite isotopic characterization at concentrations of 0.02 μmol L^{-1} or higher (Jiang et al., 2026). These analytical limitations likely contribute to the still limited application of nitrite isotope measurements in environmental studies despite their considerable interpretative potential.”

Paragraph added in the concluding section: “Although nitrite isotope measurements offer strong mechanistic insight, their application is constrained by low concentrations and analytical challenges. Recent advances in high-sensitivity methods are expanding their feasibility in natural systems. Nonetheless, these limitations continue to restrict their widespread use in environmental studies”.

Response to Referee 2

> *I appreciate the effort by the authors to reposition nitrite as a central node governing nitrogen fate across redox gradients. The conceptual framework is interesting and potentially valuable for linking microbial metabolism, isotope dynamics, and nitrogen loss pathways.*
>
> *That said, I found some sections repetitive and occasionally overly abstract, which made the manuscript difficult to follow at times. Several lines and concepts would benefit from clearer wording and additional explanation, particularly for readers who may be less familiar with isotope systematics or nitrite turnover dynamics. The manuscript would also benefit from incorporating more concrete environmental examples to contextualize better and illustrate the conceptual arguments being made.*
>
> *A major point that I think deserves stronger discussion is the practical limitation associated with natural-abundance nitrite isotope approaches. Although the manuscript advocates for shifting focus from accumulated pools to nitrite turnover and coupling, these approaches are inherently biased toward environments and transition zones where nitrite accumulates to detectable concentrations. In many natural systems, nitrite concentrations remain below detection despite active nitrogen cycling, owing to extremely rapid turnover and tight coupling between production and consumption processes. As a result, many oxic-anoxic transition zones are spatially narrow, transient, and analytically challenging to resolve. This limitation is important because it constrains where nitrite natural-abundance isotope techniques can realistically be applied. I therefore think the manuscript would benefit from a more explicit discussion of current analytical detection limits, the concentration ranges typically required for robust d15N and d18O measurements of nitrite, and future methodological developments aimed at improving sensitivity toward nanomolar-level nitrite detection and isotope analysis.*

We thank Reviewer 2 for the careful and constructive reading of our manuscript. The reviewer raises one major point, the practical limitations of natural-abundance nitrite isotope approaches in environments where nitrite does not accumulate to analytically tractable concentrations, and a series of specific comments on clarity, terminology, and structure. We fully acknowledge the analytical limitation raised and have addressed it explicitly by adding a dedicated paragraph in the concluding section. We have also acted on the large majority of specific comments, reformulating several passages to improve clarity and reduce apparent repetition, adding brief environmental examples, and correcting terminological imprecisions. On one point, the description of nitrite as the "only" inorganic nitrogen species produced under both oxidative and reductive regimes, we maintain our position with a qualification: while nitric oxide (NO) participates in both regimes, it is extremely short-lived in aqueous solution and does not accumulate to measurable concentrations. Nitrite is therefore the only stable dissolved inorganic intermediate fulfilling this dual role, and we have revised the text to make this specificity explicit. Our responses to each comment are detailed below.

Update following the Associate Editor's comments. The Associate Editor noted that both referees converged on the wording "only stable", and recommended grounding it consistently. We have therefore based the claim on structure rather than chemical stability: nitrite occupies the +III

oxidation state, the redox transit between the reductive and oxidative branches of the network. The manuscript now describes nitrite as the “principal dissolved inorganic intermediate”, and we apply this framing consistently across the abstract, introduction, and conclusions.

Specific comments

> *Lines 24–26: “Nitrogen retention, recycling and atmospheric loss are resolved at the stage of nitrite turnover, where competing pathways partition fluxes under kinetic and environmental constraints.” Not sure what the authors are getting at here. The framing feels overly complicated.*

We have reworded the sentence to “Nitrogen retention, recycling, and losses to the atmosphere are determined during nitrite turnover, where competing pathways partition fluxes according to kinetic and environmental constraints” to improve clarity.

> *Lines 41–42: Another timely study to consider, as it discusses the impact of climate change on denitrification in an aquatic environment: https://doi.org/10.1038/s41564-026-02349-9*

We thank the reviewer for drawing our attention to this study. We note that our initial manuscript was submitted on 15 April 2026, prior to the publication of Callbeck et al. on 22 May 2026, which explains its absence from the original version. We have nevertheless incorporated this reference in the revised manuscript as it provides a timely illustration of climate-driven impacts on denitrification in aquatic systems.

> *Lines 55–62: “Nitrite is the only inorganic nitrogen species produced by both oxidative and reductive processes…” I agree that nitrite plays a central role in the nitrogen cycle, but I would suggest being a little cautious with the wording here. Nitric oxide (NO) also participates in both oxidative and reductive branches of the nitrogen cycling, and is an even more reactive and transient intermediate that is often difficult to measure. So while I agree with the overall point being made, describing nitrite as the “only” inorganic nitrogen species linking these processes may be somewhat overstated.*

We thank the reviewer for this important observation. Nitric oxide (NO) does indeed participate in both oxidative and reductive branches of the nitrogen cycle. However, NO is extremely short-lived in aqueous solution, with a half-life of seconds in the presence of dissolved oxygen, and does not accumulate to measurable concentrations under environmental conditions. It therefore cannot function as a diagnosable intermediate for natural-abundance isotope approaches. We note that this issue was also raised by Reviewer 1. We have introduced the qualifiers "stable" and "dissolved" to make explicit that the uniqueness of nitrite resides in its being the only dissolved inorganic intermediate that is both stable enough to accumulate and produced under both oxidative and reductive regimes — a combination that NO does not fulfil.

Update following the Associate Editor’s comments. In the second round of revision, the Associate Editor noted that “stable” sits uneasily against our own statement that nitrite is chemically labile. We have therefore grounded the distinction in structure rather than chemical stability: nitrite occupies the +III oxidation state, the redox transit between the reductive and

oxidative branches of the network. The sentence now reads (P3 L63-76): “The central role of nitrite follows from its position in the reaction network. It occupies the +III oxidation state, midway between ammonium and nitrate., and every transformation linking oxidative and reductive branches passes through it. (Burgin & Hamilton, 2007; Lam & Kuypers, 2011; Kraft et al., 2014). Nitric oxide (NO) also participates in both branches. Its half-life of seconds in aqueous solution keeps its concentration below measurable levels under environmental conditions, which leaves nitrite as the single dissolved intermediate accessible to direct observation. No other inorganic nitrogen compound combines this dual role of convergence and divergence.”

Update following the Associate Editor’s comments. In the second round of revision, the Associate Editor noted that “stable” sits uneasily against our own statement that nitrite is chemically labile. We have therefore grounded the distinction in structure rather than chemical stability: nitrite occupies the +III oxidation state, the redox transit between the reductive and oxidative branches of the network. The sentence now reads: “Despite nitric oxide also participating in both oxidative and reductive transformations, nitrite is the principal dissolved inorganic intermediate linking the two domains: it occupies the +III oxidation state, the obligatory redox transit between the reductive and oxidative branches of the network, and — unlike the extremely short-lived NO — can accumulate to measurable concentrations. It is the obligatory precursor to downstream nitrate, ammonium and gaseous nitrogen products.”

> *Lines 63–64: “Current diagnostics rely on concentration measurements of nitrate, ammonium and gaseous products.” A bit repetitive.*

We agree that this sentence was partly redundant with the preceding argument. We have merged it with the following sentence and added a brief environmental example to ground the argument concretely, addressing both this comment and the reviewer's request for illustration at lines 80-90 of P3.

The new sentence is: “Concentration measurements of nitrate, ammonium and gaseous products integrate multiple processes, masking mechanistic controls on pathway partitioning: similar nitrate concentrations can arise from fundamentally different combinations of nitrification, denitrification and DNRA (e.g. in riparian zones where both processes operate simultaneously), leading to divergent outcomes in nitrogen retention and N_2O emissions.”

> *Line 64: “metrics” Did you mean nutrients? Not sure what is meant by metrics?*
>
> *Lines 64–67: “These metrics integrate multiple processes, masking mechanistic controls on pathway partitioning…” The sentence is a bit vague. Could the authors provide more clarity by illustrating an environmental example?*

We agree that "metrics" was ambiguous and that the sentence was partly redundant with the preceding argument. We have merged lines 63–67 into a single sentence, replacing "metrics" with an explicit reference to "concentration measurements", and have added a brief environmental example (riparian zones with simultaneous nitrification and denitrification) to illustrate how similar nitrate pool sizes can arise from fundamentally different internal process configurations, addressing both comments simultaneously.

Lines 70–71: "its isotopic composition records concurrent production and consumption…" To provide a bit of pushback here, it is precisely because nitrite is simultaneously produced and consumed that analyzing nitrite using natural abundance isotopes becomes very complicated. This may be worth acknowledging in this passage.

The simultaneous occurrence of production and consumption does indeed complicate isotopic interpretation. This complexity is precisely what motivates the dual $\delta^{15}N$–$\delta^{18}O$ framework developed in the dedicated section. We have added a brief acknowledgement of this interpretative challenge at this location and directed the reader to the relevant section.

Lines 71–72: "Recent methodological advances…" Is it still appropriate to describe this as "recent" if the method was reported in 2005?

We agree that describing a 2005 method as "recent" is no longer appropriate. We have replaced "recent methodological advances" with "available methods", which better reflects the current state of the field while retaining the references to the original and most recent analytical developments.

Lines 73–74: "Oxygen isotopes are partially modified by exchange with water, increasing the diagnostic importance of d15N." For the uninitiated reader, this likely requires further explanation.

We have expanded this sentence to briefly explain the mechanism of oxygen isotope exchange for readers less familiar with isotope systematics. The full treatment of this phenomenon is provided in the Isotopic constraints section.

The sentence is now: "Oxygen isotopes of nitrite are partially reset due to equilibrium with oxygen isotopes of ambient water, modifying the primary biological signal." P4 L97-100.

Lines 78–79: "a basis accessible only when nitrite is treated as a control point rather than a transient residual." Not sure what the authors mean by treating nitrite as a "control point." This needs additional elaboration.

We have modified the sentence for clarification "This basis relies on considering nitrite as a central component of the nitrogen cycle rather than a transient residual.".

Line 103: "outcomes" Perhaps "end points" would be clearer?

We agree that "outcomes" was insufficiently precise in this context. We have replaced it with "fates", which better reflects the biogeochemical framing of the manuscript.

Line 107: "this configuration is redistribution, not accumulation." This is a bit vague. Also, what exactly is meant by "this configuration"?

Line 108: "flux junction" This term could benefit from further clarification.

Lines 108–109: Nitrite operates as a flux junction: its concentration reflects the balance between upstream formation and downstream consumption. It's a bit repetitive.

We have clarified the antecedent of "this configuration" by replacing it with "this convergence–divergence architecture", referring explicitly to the dual role of nitrite described in the preceding

paragraph. We have also expanded the definition of "flux junction" inline to make the term self-explanatory for readers less familiar with reaction network terminology.

> *Lines 109–115: A lot of this comes across as repetitive and reiterates the same concepts multiple times.*

We have addressed the apparent repetition in two ways. First, we removed the closing sentence of the "Branching node" section ("The fate of reactive nitrogen is determined at the stage where nitrite flux is partitioned among competing biological and abiotic transformations"), which reiterated points already made. Second, we added a transitional sentence at the opening of the "Kinetic control" section to make explicit the logical progression from the structural argument (nitrite as the reaction network node) to the kinetic argument developed in this section (gross production and consumption rates as the determinants of flux direction and magnitude). The two sections address different levels of analysis — structural and kinetic — and we hope this transition now makes that distinction clear.

> *Lines 118–122: "The direction and magnitude of nitrogen redistribution…" Again, this feels like the same concept discussed above.*

This comment is addressed by the transitional sentence added at the opening of the "Kinetic control" section in response to the preceding comment. The apparent repetition reflects the absence of an explicit link between the structural argument of the "Branching node" section and the kinetic argument developed here. We believe the added transition now clarifies that these two sections operate at different levels of analysis.

> *Lines 123–128: "Residence time at the nitrite stage…" Could the authors point to a concrete environmental example of this?*

We have modified the sentence to “Accumulation and persistence along time of nitrite”, for clarification. We have also included “see Sensitivity across environmental gradients section”, because examples are provided there.

> *Line 129: "Gaseous nitrogen production…" I would suggest explicitly stating the two gases being discussed upfront — i.e., dinitrogen (N2) and nitrous oxide (N2O).*

We have explicitly stated the two gases.

> *Line 129: "the most" I do not fully understand why this is framed as "the most" important aspect of nitrite turnover. Perhaps the authors mean from a biogeochemical perspective. From an environmental perspective, nitrite oxidation to nitrate — thereby sustaining denitrification pathways — is also critically important. I would suggest dropping "the most."*

We agree that framing gaseous nitrogen production as "the most consequential" outcome is an overstatement — nitrite oxidation back to nitrate, which sustains nitrification and fuels further denitrification, is equally important from a biogeochemical standpoint. We have replaced "the most consequential" with "a relevant" to reflect this more balanced view.

> *Line 130: "biological" I might refer to these processes as "microbial" rather than "biological."*

We have replaced "biological" with "microbial" as suggested.

Line 132: “electron supply” Could you be a bit more specific?

We have specified "organic donor supply (such as organic matter)" in place of the more generic "electron supply" to improve precision, as organic carbon is the primary electron donor driving complete denitrification to N_2 in most environmental settings.

Line 132: “completion of the sequence and” Could be worded to favor complete nitrate reduction to N2.

We respectfully note that the current phrasing already conveys this meaning. "Completion of the sequence" refers explicitly to the stepwise reduction from nitrite through NO and N_2O to N_2, and "N_2 dominance" directly expresses the outcome the reviewer requests. We have retained this formulation as it captures both the mechanistic process (sequential reduction) and its biogeochemical outcome (N_2 as the dominant product) more precisely than a simpler reference to "complete nitrate reduction to N_2" would.

Lines 140–142: “indirectly shaping N2O production in mixed-metabolism environments…” Anammox also directly contributes to N2 production using nitrite, which would also fit into the importance of nitrite. The connection of anammox to nitrite and linking this to N2O is a bit of a stretch. Why not connect anammox to nitrogen loss via N2 production?

We thank the reviewer for this correction. We agree that the primary biogeochemical significance of anammox at the nitrite node is its competition with denitrifiers for nitrite, which directly governs the partitioning between N_2 and N_2O production. We have reformulated this sentence to place this competition at the center, while retaining the implication for N_2O in mixed-metabolism environments.

Line 142: “the speciation” Not sure I understand this, might need rewording

We have replaced "the speciation of nitrogen loss" with "the relative production of N_2 versus N_2O" for clarity.

Line 147: “Stable isotope measurements…” Perhaps specify “stable isotope measurements of nitrite” for clarity.

We have added "of nitrite" to avoid any ambiguity about which isotope measurements are being referred to at the opening of this section.

Lines 209-211: Might be worth specifically referring to the oxic-anoxic transition zones.

We have specified "oxic-anoxic transition zones" in place of the more generic "redox transition zones" as suggested.

Lines 212-213: “In fully oxic environments, nitrite is produced during ammonia oxidation and rapidly oxidized to nitrate, reflecting tight coupling between nitrification steps (Casciotti, 2016).” I think an important practical limitation should be acknowledged here. In many fully oxic environments, nitrite turnover is so rapid that nitrite concentrations may remain below detection or at levels insufficient for natural-abundance isotope analyses. For example, in stratified water column systems, we often observe elevated nitrate concentrations alongside

undetectable nitrite, despite clear evidence for active ammonium oxidation based on 15NH4+ tracer-rate experiments and metagenomic data. Thus, the absence of measurable nitrite does not imply the absence of nitrification activity. This highlights one of the major challenges of using natural-abundance nitrite isotopes to assess nitrogen cycling: the approach is only feasible when sufficient nitrite accumulates for analysis, which often restricts applications to narrow oxic–anoxic transition zones where nitrite transiently accumulates. It may therefore be helpful for the review to discuss the current analytical limits for natural-abundance nitrite isotope measurements. What nitrite concentrations are presently required for robust d15N and d18O analyses, and what represents the current cutting edge in analytical sensitivity? More broadly, it would be interesting to discuss whether the field is moving toward the ability to quantify nitrite isotopes at nanomolar concentrations, analogous to recent advances enabling oxygen measurements at picomolar levels. Without such advances, natural-abundance NO2- isotope approaches may remain restricted to relatively localized environments with micromolar nitrite accumulation, which can be spatially and temporally very constrained.

Lines 218-220: "2016). These zones are recognized as hotspots of N2O production, consistent with the central role of nitrite in regulating the N2O:N2 ratio (Butterbach-Bahl et al., 2013; Babbin et al., 2020)." One challenge worth acknowledging here is that many environmental hotspots of N2O and N2 production occur under conditions where nitrite itself remains below detection. In such systems, strong nitrogen turnover may clearly be occurring, yet the direct application of natural-abundance nitrite isotope approaches becomes extremely difficult or even impossible. This limitation is fundamentally tied to analytical sensitivity at multiple stages: first, the chemical detection of nitrite itself; second, the efficiency and sensitivity of converting NO2- to an analyzable gas species (e.g., N2O or N2, depending on the analytical approach); and finally, the detection limits and precision of the isotope ratio mass spectrometer. As a result, although nitrite may mechanistically regulate these processes, the practical ability to use nitrite natural-abundance isotopes as a tracer is often restricted to environments where nitrite transiently accumulates to sufficiently high concentrations for robust analysis.

Line 241: "from accumulation to coupling…" As mentioned above, an important practical limitation is that natural-abundance nitrite isotope approaches are inherently biased toward environments and transition zones where nitrite accumulates to detectable concentrations. In many systems, these zones are spatially narrow, transient, or characterized by NO2- concentrations below analytical detection limits despite active nitrogen cycling. This analytical constraint may be worth acknowledging more explicitly when advocating for a shift from accumulation-based to coupling-based interpretations of nitrogen cycling.

We thank the reviewer for raising this important practical limitation, which is indeed central to an honest appraisal of the framework we advocate. We fully agree that natural-abundance nitrite isotope approaches are currently constrained to environments where nitrite accumulates to analytically tractable concentrations, and that many oxic-anoxic transition zones, precisely the settings where nitrite turnover is most intense, are spatially narrow, transient, and often characterized by nitrite concentrations below current detection limits. We have addressed this limitation explicitly by adding a dedicated paragraph in the Isotopes section (and briefly in the

concluding section). We believe that acknowledging these constraints directly, rather than dispersing brief caveats across multiple sections, provides a more coherent and honest treatment of the gap between the conceptual framework and its current analytical reach.

The paragraph added in the Isotopes section: “Despite their strong mechanistic potential, nitrite isotope measurements are only feasible when sufficient nitrite accumulates for analysis and remain analytically challenging. Nitrite is chemically unstable and may undergo rapid oxidation or reduction during sampling and storage, potentially altering its isotopic composition before analysis. Reliable preservation therefore requires rapid processing and carefully controlled storage conditions, often under alkaline conditions that minimize transformation rates. In many terrestrial and aquatic systems, low nitrite concentrations restrict isotope analyses to zones where nitrite transiently accumulates. This emphasizes the need for continued development of high-sensitivity analytical approaches (Lewicka-Szczebak et al., 2021; Deb and Lewicka-Szczebak, 2025). For instance, a recent study developed a method for freshwater samples by coupling anion-exchange resin preconcentration with the azide reduction method for nitrite isotopic characterization at concentrations of 0.02 μmol L^{-1} or higher (Jiang et al., 2026). These analytical limitations likely contribute to the still limited application of nitrite isotope measurements in environmental studies despite their considerable interpretative potential.”

The paragraph added in the concluding section: “Although nitrite isotope measurements offer strong mechanistic insight, their application is constrained by low concentrations and analytical challenges. Recent advances in high-sensitivity methods are expanding their feasibility in natural systems. Nonetheless, these limitations continue to restrict their widespread use in environmental studies”.