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.” Lines 57-59. We have also explained in detail the role of hydroxylamine in the caption of Figure 1.

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. Lines 65-66.

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. Lines 77-78.

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. Lines 134-135.

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. Lines 205-227.

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. Lines 291-294.

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 (Lines 205-218): “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 (Lines 298-302): “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”.

Ideas and perspectives: Nitrite turnover controls nitrogen fate across redox gradients

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Abstract

Reactive nitrogen fate in natural systems remains difficult to predict because pathway partitioning occurs at the stage of nitrite turnover, where rapid and tightly coupled production and consumption processes obscure the underlying fluxes. Concentration-based assessments emphasize the dominant pools — nitrate and ammonium — and pathway divergence is determined at the stage of nitrite turnover, independently of pool size. Nitrite is the only inorganic nitrogen species produced under both oxidative and reductive regimes and the obligatory precursor to all downstream dissolved and gaseous products. Because nitrite rarely accumulates, it has often been treated as a transient intermediate of limited interpretive value. This apparent invisibility reflects rapid, tightly coupled turnover and does not indicate functional insignificance. Nitrogen retention, recycling and losses to the atmosphere are determined during nitrite turnover, where competing pathways partition fluxes according to kinetic and environmental constraints.

Observed concentrations integrate formation and consumption into a net signal that masks opposing fluxes when internal cycling is rapid. Coupled $\delta^{15}N$–$\delta^{18}O$ measurements of nitrite constrain simultaneous production and consumption and differentiate biological from abiotic pathways. Partial oxygen isotope exchange with water increases the diagnostic primacy of $\delta^{15}N$ in resolving hidden turnover. Centering nitrogen-cycle interpretation on nitrite dynamics and isotopic expression across redox gradients from oxic soils to oxygen minimum zones, provides a mechanistic basis for predicting nitrogen budgets, N_2O emissions, and ecosystem sensitivity to increasing redox variability under climate change and land-use intensification.

Introduction

The nitrogen cycle regulates ecosystem productivity, water quality and climate. Anthropogenic perturbations have profoundly altered nitrogen fluxes across terrestrial and aquatic systems, leading to nitrate (NO_3^-) contamination, eutrophication, and enhanced emissions of nitrous oxide (N_2O), a potent greenhouse gas and ozone-depleting substance (Vitousek et al., 1997; Galloway et al., 2004; Gruber & Galloway, 2008; Callbeck et al., 2026). Uncertainty persists regarding the mechanisms that determine whether reactive nitrogen is retained within ecosystems or transferred to the atmosphere.

Conceptual representations of the nitrogen cycle have traditionally emphasized stable pools — ammonium (NH_4^+) and nitrate (NO_3^-) — providing a coherent basis for large-scale budget analyses (Canfield et al., 2010). However, the location within the reaction sequence at which pathway divergence is determined remains unresolved.

In reaction networks governed by rapid kinetics and tight coupling, the apparent invisibility of intermediates reflects rapid turnover rather than limited functional relevance. Nitrite (NO_2^-) exemplifies this conceptual blind spot. Described as an ephemeral intermediate that does not accumulate under steady-state conditions (Heil et al., 2016; Wrage et al., 2001), it is often omitted from conceptual frameworks or treated implicitly. This assumption is not consistent with the structure of the nitrogen cycle reaction network.

Nitrite is the central stable dissolved intermediate linking oxidative and reductive nitrogen transformations and the obligatory precursor to downstream nitrate, ammonium and gaseous nitrogen products (Burgin & Hamilton, 2007; Lam & Kuypers, 2011; Kraft et al., 2014). Despite NO also participates in both oxidative and reductive branches of the nitrogen cycle it is extremely short-lived in aqueous solution and does not accumulate to measurable concentrations under environmental conditions. No other inorganic nitrogen compound combines this dual role of convergence and divergence. The fate of nitrogen at this junction is governed by the balance between nitrite-producing and nitrite-consuming processes under specific redox, kinetic and environmental constraints (Firestone & Davidson, 1989; Denk et al., 2017; Deb et al., 2024).

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.

Stable isotope measurements provide direct constraints on these hidden dynamics. Nitrite integrates oxidative and reductive fluxes; its isotopic composition records concurrent production and consumption even when net concentrations remain unchanged, though disentangling these overlapping signals requires the dual $\delta^{15}N$–$\delta^{18}O$ approach described in

detail in the Isotopic constraints section below. Available methods advances permit direct determination of natural-abundance $\delta^{15}N$ and $\delta^{18}O$ in nitrite (McIlvin & Altabet, 2005; Sebilo et al., 2019; Deb & Lewicka-Szczebak, 2025; Hu et al., 2026). Oxygen isotopes of nitrite are partially reset due to equilibrium with oxygen isotopes of ambient water, modifying the primary biological signal and thereby increasing the diagnostic importance of $\delta^{15}N$.

Resolving nitrogen fate requires shifting analytical focus from accumulated pools to the intermediate at which pathway divergence is decided. The reaction network structure, kinetics of nitrite turnover, and stable isotope constraints together provide a mechanistic basis for predicting nitrogen budgets and N_2O emissions. This basis relies on considering nitrite as a central component of the nitrogen cycle rather than a transient residual.

Nitrite as the structural branching node

The central role of nitrite emerges directly from the reaction network architecture (Fig. 1). Nitrite does not constitute a regulatory control point in a biochemical sense, but rather the reaction network node at which pathway partitioning is resolved. Under oxic conditions, nitrite is produced during ammonium oxidation by ammonia-oxidizing bacteria and archaea and subsequently oxidized to nitrate, or diverted into alternative pathways depending on oxygen availability and enzyme kinetics (Casciotti, 2016). In comammox organisms, both oxidation steps occur within a single metabolic framework (Daims et al., 2015; van Kessel et al., 2015), further constraining accumulation while maintaining high gross turnover.

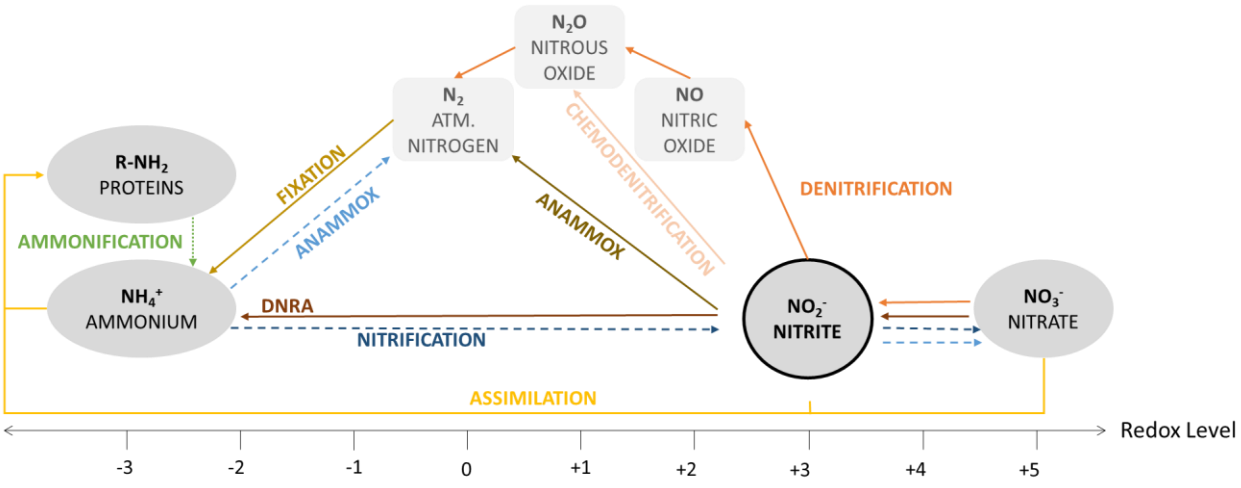

Figure 1. Nitrite-based nitrogen cycling reactions across redox gradients. Oxidative pathways are presented in blue arrows, reductive ones in orange/brown and those involving organic matter in yellow/green. To simplify the scheme, hydroxylamine has not been included in the figure. Hydroxylamine is also involved in oxidative and reductive pathways. It can be produced during ammonia oxidation and nitrite reduction. It is primarily oxidized by nitrifying microorganisms to nitrite, with minor

side production of nitric oxide and nitrous oxide, and it can be reduced to ammonium. However, it is highly reactive, it is usually rapidly oxidized before accumulating in the environment, limiting its influence at the ecosystem scale.

Under oxygen-limited or anoxic conditions, nitrite is produced during nitrate reduction and partitioned among denitrification, DNRA, anammox, and abiotic reduction. Each pathway channels nitrite toward distinct fates, from nitrogen retention to irreversible gaseous loss (Zumft, 1997; Lam & Kuypers, 2011). Abiotic reactions extend this network: reduction of nitrite by ferrous iron generates NO and N_2O independently of enzymatic control, competing with biological pathways (Jones et al., 2015; Grabb et al., 2017; Robinson et al., 2021).

The defining characteristic of this convergence-divergence architecture is redistribution, not accumulation. Nitrite operates as a flux junction, a node at which inputs from multiple upstream pathways are portioned among competing downstream transformations, so that its concentration reflects the balance between upstream formation and downstream consumption, while its turnover rate determines the direction and magnitude of nitrogen transfer. In spatially heterogeneous environments, nitrite produced in one microdomain may be consumed in an adjacent zone within short diffusion distances (Firestone & Davidson, 1989). Accumulation arises primarily when this coupling is disrupted by kinetic limitation, redox fluctuation, or imbalance in electron donor and acceptor supply.

Kinetic control of nitrite turnover and gaseous nitrogen speciation

If the structural position of nitrite determines where pathway divergence occurs, the kinetic balance between gross production and gross consumption determines how. The direction and magnitude of nitrogen redistribution at the nitrite node depend on the kinetic balance between gross production and gross consumption. When these proceed at comparable rates, net concentration change approaches zero while flux through the intermediate remains substantial; pool size therefore provides limited information about pathway activity (Margalef-Marti et al., 2026). Such tightly coupled nitrite turnover has recently been observed in nitrate-rich groundwater systems using combined isotope and microbial approaches (Deb et al., 2025).

Accumulation and persistence along time of nitrite is determined by the degree of kinetic coupling between sequential reactions. Tight coupling in oxic systems constrains accumulation and shortens residence time; disruption by fluctuating oxygen supply, transport limitation, or electron donor imbalance prolongs residence time and increases the probability of accumulation (see Sensitivity across environmental gradients section). Shifts in

environmental conditions reorganize flux distribution at the nitrite node even when nitrate or ammonium pools exhibit minimal change.

Gaseous nitrogen (i.e., NO, N_2O and N_2) production represents a relevant downstream outcome of nitrite turnover. In microbial denitrification, the relative gross rates of successive reductions from nitrite to NO, N_2O, and ultimately N_2, determine the N_2O:N_2 ratio. Sustained electron donor supply (such as organic matter) and active nitrous oxide reductase favour completion of the sequence and N_2 dominance; partial decoupling enhances expression of intermediate products, particularly N_2O (Firestone & Davidson, 1989; Zumft, 1997; Lewicka-Szczebak et al., 2020). During oxygen-limited nitrification, nitrite generated from ammonium oxidation may be partially reduced within ammonia oxidizers, producing N_2O under fluctuating oxygen conditions (Wrage et al., 2001). Abiotic reduction of nitrite by ferrous iron and reduced mineral phases generates NO and N_2O independently of enzymatic control (Jones et al., 2015; Grabb et al., 2017).

Although N_2O is not a primary product of canonical anammox metabolism — hydrazine oxidation yields N_2 directly — anammox competes with denitrifiers for the available nitrite pool, thereby influencing the partitioning of nitrite between N_2-producing and N_2O-producing pathways in mixed-metabolism environments (Kartal et al., 2011). Therefore, the N_2O:N_2 ratio, and the relative production of N_2 versus N_2O more broadly, is controlled by turnover intensity and kinetic coupling at the nitrite stage rather than by pool size.

Isotopic constraints on gross nitrite turnover

Stable isotope measurements of nitrite provide direct constraints on nitrogen transformations at the stage where flux redistribution occurs: the nitrite node. Isotopic composition responds to gross production and gross consumption rather than to net pool change alone. Constant concentration does not imply constant $\delta^{15}N$: progressive isotopic shifts may occur under steady-state pool conditions, revealing turnover intensity undetectable in concentration data (Fig. 2).

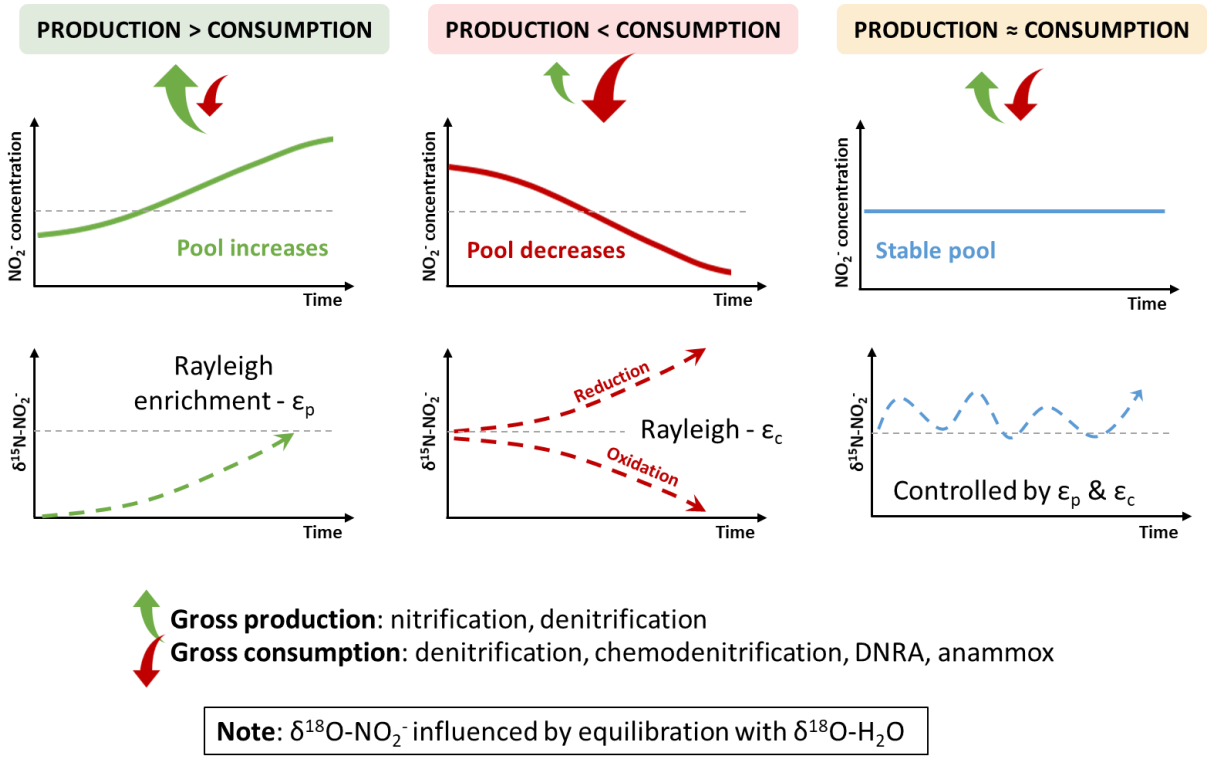

Figure 2. Isotopic expression of gross nitrite turnover under contrasting production–consumption regimes. Top panels show nitrite concentration ([NO_2^-]) as a function of time, reflecting the net balance between gross production (P) and gross consumption (C): accumulation when P > C, depletion when P < C, and quasi-steady state when P ≈ C despite high internal fluxes. Bottom panels illustrate conceptual trajectories of $\delta^{15}N(NO_2^-)$. In contrast to concentration, isotopic composition responds to gross turnover rather than net change. $\delta^{15}N(NO_2^-)$ evolves according to the isotopic signatures of contributing sources and the combined effects of isotope fractionation during production (εP) and consumption (εC). The direction of isotopic change is therefore process-dependent and not universal (e.g. inverse isotope effects during nitrite production by nitrification). Under conditions of balanced production and consumption (P ≈ C), $\delta^{15}N(NO_2^-)$ may drift despite constant concentration, reflecting ongoing gross turnover. Oxygen isotope signals ($\delta^{18}O$) may be partially overprinted by exchange with water, increasing the diagnostic primacy of $\delta^{15}N$.

Each pathway imposes a characteristic kinetic isotope fractionation. During ammonia oxidation, inverse nitrogen isotope effects may enrich newly formed nitrite relative to its ammonium source (Casciotti et al., 2003; Santoro & Casciotti, 2011). During nitrite oxidation to nitrate, preferential removal of lighter isotopes enriches the residual pool in both ^{15}N and ^{18}O (Casciotti, 2009; Buchwald et al., 2012). Reductive pathways generate distinct fractionation patterns associated with nitrite reductase activity (Brunner & Bernasconi, 2005; Casciotti et al., 2010). Abiotic reduction by ferrous iron produces additional nitrogen isotope effects governed by surface-mediated electron transfer (Jones et al., 2015; Grabb et al., 2017). Overlapping fractionation factors across biological and abiotic pathways preclude simple end-member mixing and require integration with environmental and redox context. The direction of $\delta^{15}N\text{-}NO_2^-$ evolution is not universal and depends on the relative contributions of concurrent

production and consumption pathways, their associated isotope effects (εP, εC), and the isotopic composition of the source substrate.

Oxygen isotopes introduce an additional dimension. Exchange between nitrite oxygen atoms and ambient water may proceed on timescales comparable to biological turnover (Buchwald & Casciotti, 2010; Casciotti et al., 2010; Granger & Wankel, 2016), attenuating the independence of $\delta^{18}O$ as a pathway tracer. Nitrogen isotopes do not undergo analogous exchange, preserving sensitivity to pathway-specific fractionation. Under conditions of significant oxygen isotope exchange, $\delta^{15}N$ provides the more robust constraint on gross turnover.

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.

Isotopic signatures of nitrite propagate to gaseous products. The bulk $\delta^{15}N$ of N_2O reflects fractionation associated with nitrite reductases and kinetic coupling among successive reduction steps. Intramolecular ^{15}N site preference (SP) provides additional mechanistic resolution: because SP is largely independent of the isotopic composition of precursor nitrite, it records enzyme-specific reaction pathways involved in N_2O formation (Brunner & Bernasconi, 2005; Toyoda et al., 2017). Variations in SP discriminate among nitrifier-denitrification, canonical denitrification, and partial reduction sequences downstream of nitrite. Isotopomer signatures must account for subsequent N_2O reduction and mixing, which may overprint primary fractionation signals (Toyoda et al., 2017; Lewicka-Szczebak et al., 2020).

Sensitivity across environmental gradients

Environmental systems differ not in whether nitrite turnover operates, but in how sensitively flux redistribution at the nitrite node responds to perturbation. Sensitivity is highest where

oxidative and reductive metabolisms overlap at oxic-anoxic transition zones, and attenuates where reaction coupling is constrained to a single redox regime. Small shifts in oxygen supply, electron donor availability, or hydrological residence time can reorganize pathway partitioning at the nitrite node without producing detectable changes in bulk nitrogen pools. The nitrogen cycle is therefore most vulnerable to mechanistic misinterpretation precisely where nitrite turnover is most intense.

In fully oxic environments, nitrite is produced during ammonia oxidation and rapidly oxidized to nitrate, reflecting tight coupling between nitrification steps (Casciotti, 2016). Redox transition zones — oxic–anoxic interfaces in soils, sediments, riparian zones and stratified water columns — represent the most dynamic settings (Burgin et al., 2011). Simultaneous production from ammonia oxidation and nitrate reduction, combined with kinetically constrained consumption, promotes nitrite accumulation and amplifies sensitivity to environmental change (Buchwald & Casciotti, 2010; Bristow et al., 2016). These zones are recognized as hotspots of N_2O production, consistent with the central role of nitrite in regulating the N_2O:N_2 ratio (Butterbach-Bahl et al., 2013; Babbin et al., 2020). In persistently anoxic environments — deep sediments, saturated soils and oxygen minimum zones — nitrite is predominantly generated via nitrate reduction and consumed through denitrification, DNRA or anammox (Zumft, 1997; Lam & Kuypers, 2011; Ward et al., 2009; Dalsgaard et al., 2012; Kalvelage et al., 2013; Denk et al., 2017; Deb et al., 2024). Hydrological and transport processes further modulate this coupling across all settings: long residence times favor complete turnover, whereas rapid transport can decouple production from consumption, allowing accumulation or downstream export (Sebilo et al., 2006).

Nitrite as the control point of nitrogen fate

Nitrogen cycling is commonly interpreted through the distribution of dominant inorganic pools. This perspective captures accumulation and export, it does not identify where transformation trajectories are decided. The reaction network places that decision at the stage of nitrite turnover. Environmental conditions regulate this distribution by modulating the rates of nitrite production and consumption.

This reframing resolves several persistent ambiguities. Similar nitrate or ammonium concentrations can arise from fundamentally different internal configurations of production and consumption. N_2O emissions cannot be predicted from pool size alone because their magnitude depends on the completeness of reduction downstream of nitrite. Redox transition zones emerge as hotspots not because they contain larger pools, but because they intensify flux redistribution at this intermediate. A nitrite-centred framework shifts emphasis from storage to flux (Fig. 3), from accumulation to coupling, and from static pools to dynamic

turnover, linking microbial metabolism, abiotic reactivity, redox heterogeneity, and atmospheric exchange within a single mechanistic perspective. Because both production and reduction of N_2O depend on nitrite availability and turnover, resolving dynamics at this node provides a direct mechanistic link between microbial processes and climate-relevant gas fluxes.

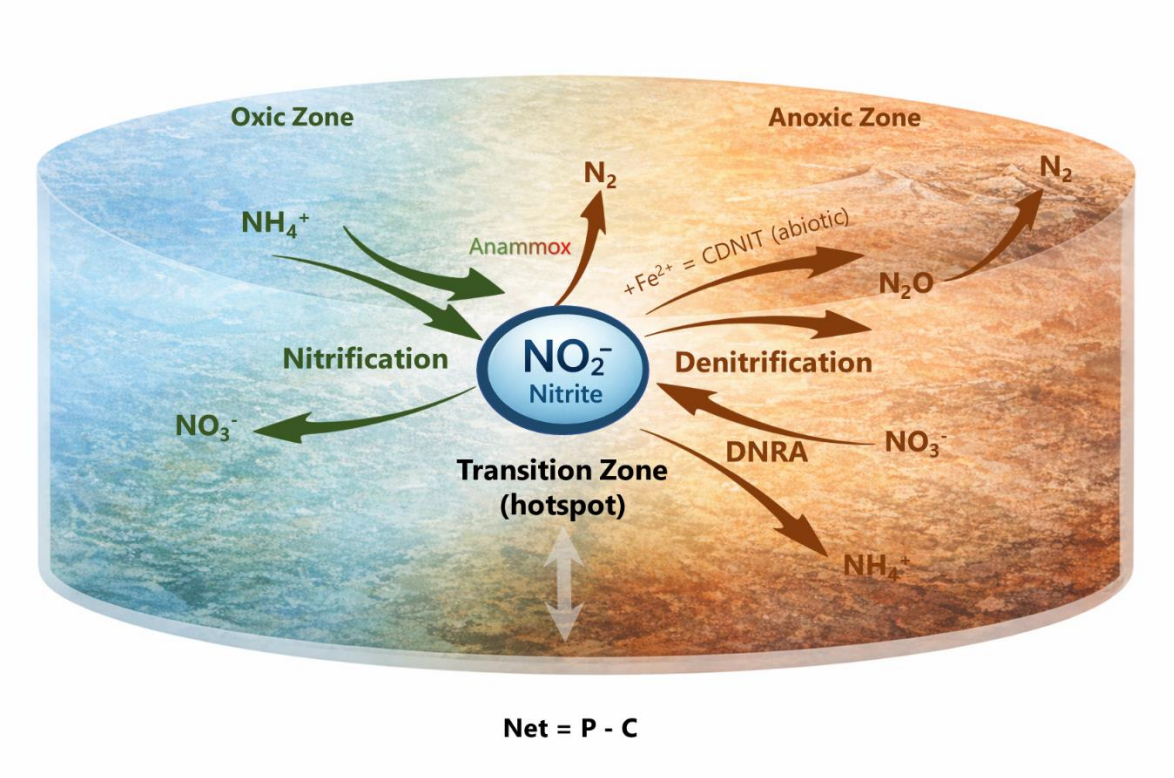

Figure 3. Conceptual synthesis positioning nitrite turnover as the control point linking redox gradients, gross flux redistribution, isotopic expression, and nitrogen fate. Nitrite integrates oxidative and reductive processes across environmental gradients. Gross production and gross consumption determine residence time and flux partitioning among retention, recycling, and gaseous loss. The $\delta^{15}N$ of NO_2^- records turnover intensity, whereas the $\delta^{18}O$ may be modified by exchange with water. Isotopic signals propagate to N_2O and inform pathway attribution. Nitrogen fate is therefore governed at the nitrite stage rather than by the size of accumulated nitrate or ammonium pools.

Concentration reflects net balance between production and consumption. $\delta^{15}N$ records the imprint of concurrent production and consumption even when pool size remains constant. Oxygen isotope exchange constrains interpretation but reinforces the diagnostic primacy of nitrogen isotopes. At the same time, the extent of oxygen exchange with water may provide information on nitrite residence times and the relative rates of biological turnover versus abiotic exchange (Buchwald and Casciotti, 2013; Lewicka-Szczebak et al., 2021). Incorporating nitrite isotopes converts an otherwise unobservable internal turnover into a measurable quantity.

Translating this framework into practice requires prioritising direct nitrite isotope measurements alongside conventional concentration measurements, particularly in redox-dynamic systems where net fluxes are most ambiguous. Dual $\delta^{15}N$–$\delta^{18}O$ approaches, combined with isotopomer analysis of N_2O, offer the most diagnostic power. 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.

At the modelling scale, representing nitrite explicitly as a state variable, rather than collapsing nitrification and denitrification into net transformations, would improve mechanistic fidelity in biogeochemical models applied to nitrogen budgets and greenhouse gas inventories. The measurement and modelling strategies that follow from this reorientation are those organised around the node where nitrogen fate is actually decided.

As environmental systems experience increasing redox variability under climate change and land-use intensification, sensitivity at the nitrite stage is likely to amplify. Understanding nitrogen cycling at Earth-system scale requires resolving where and how nitrite flux is redistributed. Without explicit consideration of turnover at this branching node, interpretations of nitrogen budgets, greenhouse gas emissions, and isotopic signals remain incomplete.

Author contribution

MS and RM jointly conceived the perspective and wrote the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Data and code availability

No new data or code were generated for this study. The perspective is based exclusively on bibliographic sources, all of which are cited in the reference list.

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