

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

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16 **Abstract**

17 Reactive nitrogen fate in natural systems remains difficult to predict because pathway
18 partitioning occurs at the stage of nitrite turnover, where rapid and tightly coupled production
19 and consumption processes obscure the underlying fluxes. Concentration-based
20 assessments emphasize the dominant pools — nitrate and ammonium — ~~and~~ while pathway
21 divergence is determined at the stage of nitrite turnover, independently of pool size. Nitrite is
22 the principal dissolved inorganic intermediate linking the oxidative and reductive branches of
23 the nitrogen cycle and ~~only inorganic nitrogen species produced under both oxidative and~~
24 ~~reductive regimes and~~ the obligatory precursor to all downstream dissolved and gaseous
25 products. Because nitrite rarely accumulates, it has often been treated as a transient
26 intermediate of limited interpretive value. This apparent invisibility reflects rapid, tightly
27 coupled turnover and does not indicate functional insignificance. Its low and frequently
28 undetectable concentration is the kinetic signature of this central position rather than evidence
29 against it: rapid coupled turnover sustains high gross flux at near-zero standing concentration.
30 Nitrogen retention, recycling and losses to the atmospheric ~~ic loss~~ are determined ~~resolved at~~
31 ~~the stage of~~ during nitrite turnover, where competing pathways partition fluxes ~~under~~ according
32 to kinetic and environmental constraints.

33 Observed concentrations integrate formation and consumption into a net signal that masks
34 opposing fluxes when internal cycling is rapid. Coupled $\delta^{15}\text{N}$ – $\delta^{18}\text{O}$ measurements of nitrite
35 constrain simultaneous production and consumption and differentiate biological from abiotic
36 pathways. Partial oxygen isotope exchange with water increases the diagnostic primacy of
37 $\delta^{15}\text{N}$ in resolving hidden turnover. However, its low concentration in natural environments can
38 pose some challenges for analysis, requiring more sensitive approaches.

39 Centering nitrogen-cycle interpretation on nitrite dynamics and isotopic expression across
40 redox gradients from oxic soils to oxygen minimum zones, provides a mechanistic basis for
41 predicting nitrogen budgets, N_2O emissions, and ecosystem sensitivity to increasing redox
42 variability under climate change and land-use intensification.

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45 Introduction

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

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

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

63 [The central role of nitrite follows from its position in the reaction network. It occupies the +III
64 oxidation state, midway between ammonium and nitrate., and every transformation is the
65 central stable dissolved intermediate linking oxidative and reductive branches passes through
66 it. nitrogen transformations only inorganic nitrogen species produced by both oxidative and
67 reductive processes, and the obligatory precursor to downstream nitrate, ammonium and
68 gaseous nitrogen products substrate for all downstream transformations: denitrification,
69 dissimilatory nitrate reduction to ammonium \(DNRA\), nitrifier-denitrification, anammox and
70 chemodenitrification](#) (Burgin & Hamilton, 2007; Lam & Kuypers, 2011; Kraft et al., 2014). [Nitric
71 oxide](#) (NO) also participates in both branches. [Its half-life of seconds in aqueous
72 solution keeps its concentration below measurable levels under environmental conditions,
73 which leaves nitrite as the single dissolved intermediate accessible to direct
74 observation. oxidative and reductive branches of the nitrogen cycle it is extremely short-lived
75 in aqueous solution and does not accumulate to measurable concentrations under
76 environmental conditions.](#) No other inorganic nitrogen compound combines this dual role of
77 convergence and divergence. The fate of nitrogen at this junction is governed by the balance
78 between nitrite-producing and nitrite-consuming processes under specific redox, kinetic and
79 environmental constraints (Firestone & Davidson, 1989; [Denk et al., 2017](#); [Deb et al., 2024](#)).
80 [Concentration measurements of nitrate, ammonium and gaseous products integrate multiple
81 processes, masking mechanistic controls on pathway partitioning: similar nitrate](#)

82 [concentrations can arise from fundamentally different combinations of nitrification,](#)
83 [denitrification and DNRA \(e.g. in riparian zones where both processes operate](#)
84 [simultaneously\), leading to divergent outcomes in nitrogen retention and N₂O emissions.](#)
85 ~~Current diagnostics rely on concentration measurements of nitrate, ammonium and gaseous~~
86 ~~products. These metrics integrate multiple processes, masking mechanistic controls on~~
87 ~~pathway partitioning: similar nitrate concentrations can arise from fundamentally different~~
88 ~~combinations of nitrification, denitrification and DNRA, leading to divergent outcomes in~~
89 ~~nitrogen retention and N₂O emissions. A framework centered on nitrite would target the step~~
90 ~~at which these pathways converge and diverge.~~

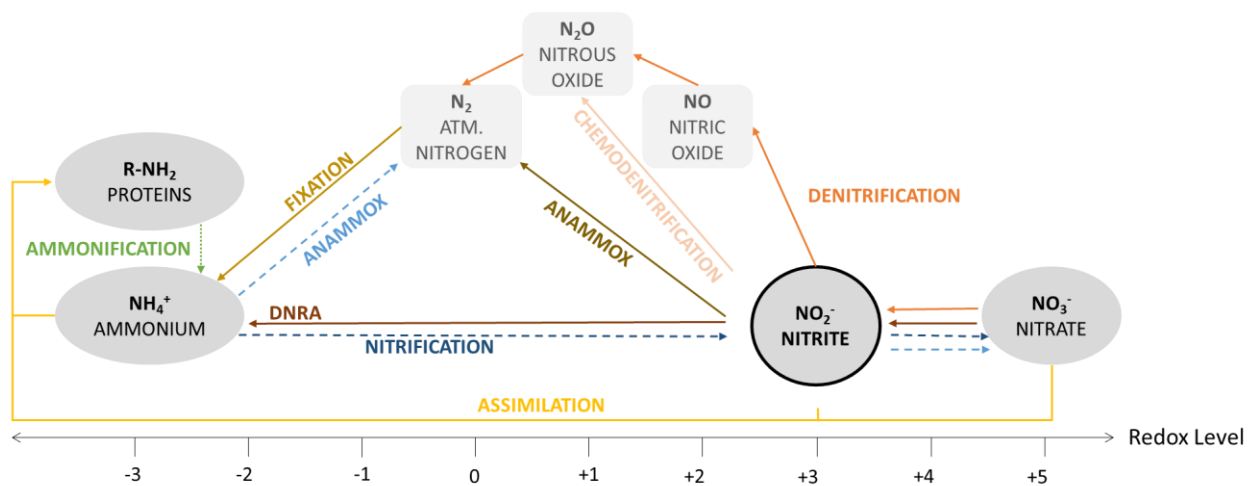
91 Stable isotope measurements provide direct constraints on these hidden dynamics. Nitrite
92 integrates oxidative and reductive fluxes; its isotopic composition records concurrent
93 production and consumption even when net concentrations remain unchanged, [though](#)
94 [disentangling these overlapping signals requires the dual \$\delta^{15}\text{N}\$ – \$\delta^{18}\text{O}\$ approach described](#)
95 [in detail in the Isotopic constraints section below.](#) ~~Available methods~~ ~~Recent methodological~~
96 advances permit direct determination of natural-abundance $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in nitrite (McIlvin &
97 Altabet, 2005; Sebilo et al., 2019; [Deb & Lewicka-Szczebak, 2025](#); [Hu et al., 2026](#)). Oxygen
98 isotopes [of nitrite](#) are partially ~~reset by back~~ ~~due to exchange~~ ~~equilibrium,~~ ~~modified by exchange~~
99 with [oxygen isotopes of ambient](#) water, ~~attenuating~~ [modifying the primary biological signal and](#)
100 [thereby](#) increasing the diagnostic importance of $\delta^{15}\text{N}$.

101 Resolving nitrogen fate requires shifting analytical focus from accumulated pools to the
102 intermediate at which pathway divergence is decided. The reaction network structure, kinetics
103 of nitrite turnover, and stable isotope constraints together provide a mechanistic basis for
104 predicting nitrogen budgets and N₂O emissions, ~~a~~ [This](#) basis ~~accessible only when~~ ~~relies on~~
105 [considering](#) nitrite ~~is treated~~ as a ~~control point~~ [central component of the nitrogen cycle](#) rather
106 than a transient residual.

107 108 109 **Nitrite as the structural branching node**

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

118



119

120 **Figure 1. Nitrite-based nitrogen cycling reactions across redox gradients.** Oxidative pathways are
 121 presented in blue arrows, reductive ones in orange/brown and those involving organic matter in
 122 yellow/green. To simplify the scheme, hydroxylamine has not been included in the figure.
 123 Hydroxylamine is also involved in oxidative and reductive pathways. It can be produced during ammonia
 124 oxidation and nitrite reduction. It is primarily oxidized by nitrifying microorganisms to nitrite, with minor
 125 side production of nitric oxide and nitrous oxide, and it can be reduced to ammonium. However, it is
 126 highly reactive, it is usually rapidly oxidized before accumulating in the environment, limiting its influence
 127 at the ecosystem scale.

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129

130 Under oxygen-limited or anoxic conditions, nitrite is produced during nitrate reduction and
 131 partitioned among denitrification, DNRA, anammox, and abiotic reduction. Each pathway
 132 channels nitrite toward distinct fates outcomes, from nitrogen retention to irreversible gaseous
 133 loss (Zumft, 1997; Lam & Kuypers, 2011). Abiotic reactions extend this network in both redox
 134 directions: reduction of nitrite by ferrous iron generates NO and N₂O independently of
 135 enzymatic control, competing with biological pathways (Jones et al., 2015; Grabb et al., 2017;
 136 Robinson et al., 2021), and oxidation of nitrite to nitrate by ligand-bound Mn(III) proceeds
 137 abiotically under both oxic and anoxic conditions (Karolewski et al., 2021; Luther et al., 2021).

138 .

139 The defining characteristic of this convergence-divergence architecture configuration is
 140 redistribution, not accumulation. Nitrite operates as a flux junction, a node at which inputs from
 141 multiple upstream pathways are portioned among competing downstream transformations, so
 142 that :-its concentration reflects the balance between upstream formation and downstream
 143 consumption, while its turnover rate determines the direction and magnitude of nitrogen
 144 transfer. In spatially heterogeneous environments, nitrite produced in one microdomain may
 145 be consumed in an adjacent zone within short diffusion distances (Firestone & Davidson,

146 1989). Accumulation arises primarily when this coupling is disrupted by kinetic limitation, redox
147 fluctuation, or imbalance in electron donor and acceptor supply.

148 ~~The fate of reactive nitrogen is determined at the stage where nitrite flux is partitioned among~~
149 ~~competing biological and abiotic transformations.~~

150

151 **Kinetic control of nitrite turnover and gaseous nitrogen speciation**

152 If the structural position of nitrite determines where pathway divergence occurs, the kinetic
153 balance between gross production and gross consumption determines how. The direction and
154 magnitude of nitrogen redistribution at the nitrite node depend on the kinetic balance between
155 gross production and gross consumption. When these proceed at comparable rates, net
156 concentration change approaches zero while flux through the intermediate remains
157 substantial; pool size therefore provides limited information about pathway activity (Margalef-
158 Marti et al., 2026). Such tightly coupled nitrite turnover has recently been observed in nitrate-
159 rich groundwater systems using combined isotope and microbial approaches (Deb et al.,
160 2025). A distinct regime arises when consumption is limited only by the rate of supply: nitrite
161 is consumed as rapidly as it is produced, so that the standing concentration itself — not merely
162 its rate of change — approaches zero even as gross flux remains intense. This is the limiting
163 case in which an active and decisive node becomes analytically invisible.

164 ~~Residence time at the~~Accumulation and persistence along time of nitrite ~~stage~~ is determined
165 by the degree of kinetic coupling between sequential reactions. Tight coupling in oxic systems
166 constrains accumulation and shortens residence time; disruption by fluctuating oxygen supply,
167 transport limitation, or electron donor imbalance prolongs residence time and increases the
168 probability of accumulation (see Sensitivity across environmental gradients section). Shifts in
169 environmental conditions reorganize flux distribution at the nitrite node even when nitrate or
170 ammonium pools exhibit minimal change.

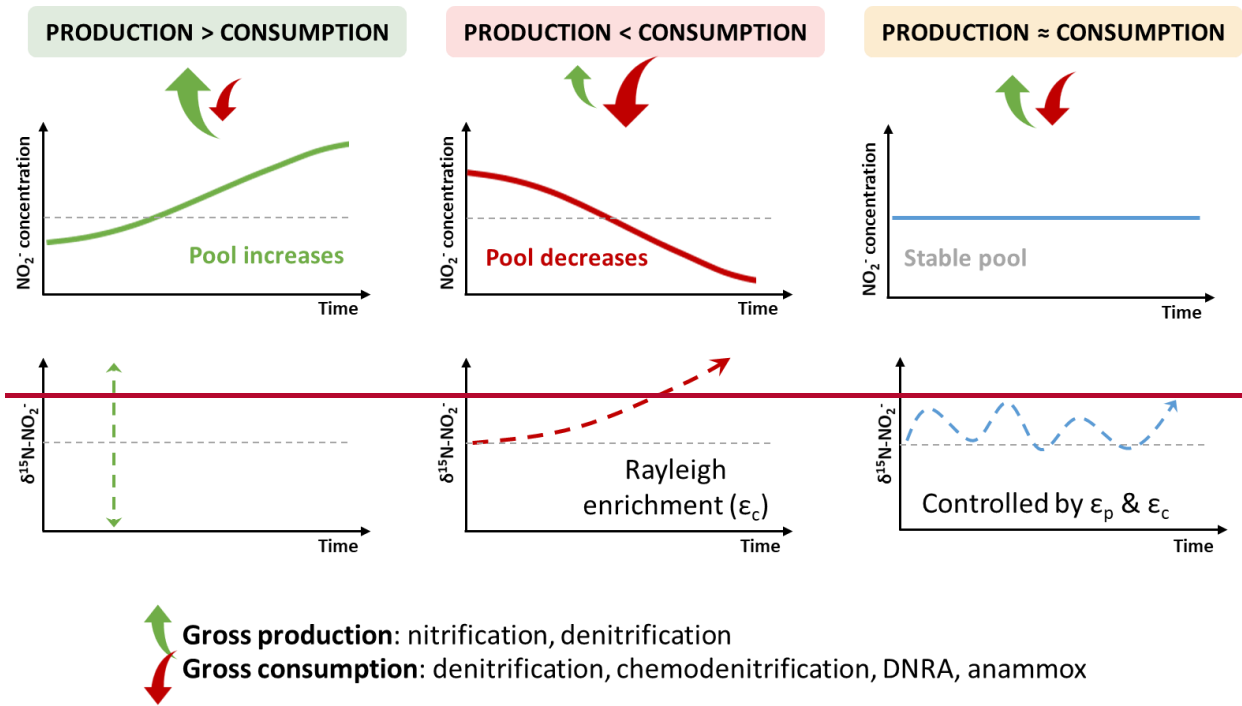
171 Gaseous nitrogen (i.e., NO, N₂O and N₂) production represents ~~a major relevant~~the most
172 ~~consequential~~ downstream outcome of nitrite turnover. In ~~microbial~~abiological denitrification,
173 the relative gross rates of successive reductions — from nitrite to NO, N₂O, and ultimately N₂
174 — determine the N₂O:N₂ ratio. Sustained electron donor supply (such as organic matter) and
175 active nitrous oxide reductase favour completion of the sequence and N₂ dominance; partial
176 decoupling enhances expression of intermediate products, particularly N₂O (Firestone &
177 Davidson, 1989; Zumft, 1997; Lewicka-Szczebak et al., 2020). During oxygen-limited
178 nitrification, nitrite generated from ammonium oxidation may be partially reduced within
179 ammonia oxidizers, producing N₂O under fluctuating oxygen conditions (Wrage et al., 2001).
180 Abiotic reduction of nitrite by ferrous iron and reduced mineral phases generates NO and N₂O
181 independently of enzymatic control (Jones et al., 2015; Grabb et al., 2017).

182 Although N_2O is not a primary product of canonical anammox metabolism — hydrazine
 183 oxidation yields N_2 directly — anammox competes with activity modifies the nitrite pool
 184 available to co-occurring denitrifiers for the available nitrite pool, thereby influencing the
 185 partitioning of nitrite between N_2 -producing and N_2O -producing pathways in and nitrifier-
 186 denitrifiers, indirectly shaping N_2O production in mixed-metabolism environments (Kartal et
 187 al., 2011). Therefore, the $N_2O:N_2$ ratio, and the relative production of N_2 versus N_2O speciation
 188 of nitrogen loss more broadly, is controlled by turnover intensity and kinetic coupling at the
 189 nitrite stage rather than by pool size.

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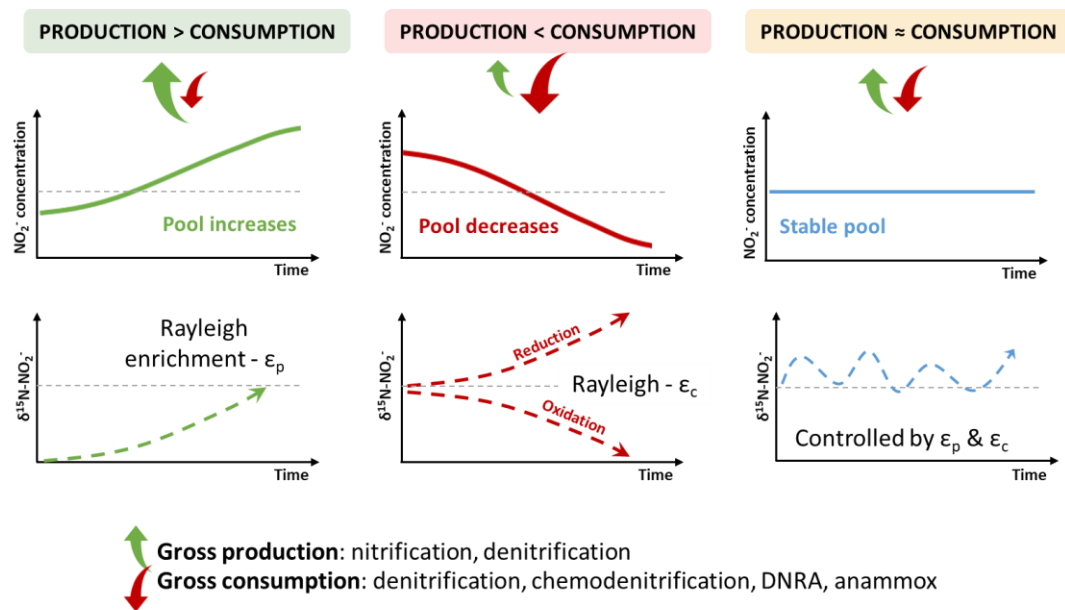
192 **Isotopic constraints on gross nitrite turnover**

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



Note: $\delta^{18}O-NO_2^-$ influenced by equilibration with $\delta^{18}O-H_2O$

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Note: $\delta^{18}\text{O}-\text{NO}_2^-$ influenced by equilibration with $\delta^{18}\text{O}-\text{H}_2\text{O}$

Figure 2. Isotopic expression of gross nitrite turnover under contrasting production-consumption regimes. Top panels show nitrite concentration ($[\text{NO}_2^-]$) 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 $P \approx C$ despite high internal fluxes. The $P \approx C$ regime spans a continuum of standing concentrations: where production and consumption balance independently, nitrite stabilises at a moderate level; where consumption is limited only by the rate of supply, nitrite is consumed as fast as it forms and its concentration approaches zero despite intense gross flux. Across this continuum, $\delta^{15}\text{N}(\text{NO}_2^-)$ continues to evolve in response to gross turnover, so that the isotopic signal persists even where the pool itself becomes analytically invisible. Bottom panels illustrate conceptual trajectories of $\delta^{15}\text{N}(\text{NO}_2^-)$. In contrast to concentration, isotopic composition responds to gross turnover rather than net change. $\delta^{15}\text{N}(\text{NO}_2^-)$ evolves according to the isotopic signatures of contributing sources and the combined effects of isotope fractionation during production (ϵ_P) and consumption (ϵ_C). The direction of isotopic change is therefore process-dependent and not universal (e.g. inverse isotope effects during nitrite production by nitrification). ~~When consumption dominates, residual nitrite may become ^{15}N -enriched if fractionation is expressed, whereas u~~ Under conditions of balanced production and consumption ($P \approx C$), $\delta^{15}\text{N}(\text{NO}_2^-)$ may drift despite constant concentration, reflecting ongoing gross turnover. Oxygen isotope signals ($\delta^{18}\text{O}$) may be partially overprinted by exchange with water, increasing the diagnostic primacy of $\delta^{15}\text{N}$.

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). Abiotic oxidation of nitrite to nitrate by ligand-bound Mn(III) imparts a large inverse nitrogen

231 [isotope effect \(\$\approx -20\%\$ \) closely resembling that of biological nitrite oxidation, with the](#)
232 [additional oxygen atom derived from water \(Karolewski et al., 2021\). This convergence of](#)
233 [biotic and abiotic fractionation factors reinforces the value of interpreting isotopic signatures](#)
234 [within environmental and redox context.](#) Overlapping fractionation factors across biological
235 and abiotic pathways preclude simple end-member mixing and require integration with
236 environmental and redox context. The direction of $\delta^{15}\text{N-NO}_2^-$ evolution is not universal and
237 depends on the relative contributions of concurrent production and consumption pathways,
238 their associated isotope effects (ϵ_P , ϵ_C), and the isotopic composition of the source substrate.
239 Oxygen isotopes introduce an additional dimension. Exchange between nitrite oxygen atoms
240 and ambient water may proceed on timescales comparable to biological turnover (Buchwald
241 & Casciotti, 2010; Casciotti et al., 2010; Granger & Wankel, 2016), attenuating the
242 independence of $\delta^{18}\text{O}$ as a pathway tracer. Nitrogen isotopes do not undergo analogous
243 exchange, preserving sensitivity to pathway-specific fractionation. Under conditions of
244 significant oxygen isotope exchange, $\delta^{15}\text{N}$ provides the more robust constraint on gross
245 turnover.

246 Isotopic signatures propagate to gaseous products. The bulk $\delta^{15}\text{N}$ of N_2O reflects fractionation
247 associated with nitrite reductases and kinetic coupling among successive reduction steps.
248 Intramolecular ^{15}N site preference (SP) provides additional mechanistic resolution: because
249 SP is largely independent of the isotopic composition of precursor nitrite, it records enzyme-
250 specific reaction pathways involved in N_2O formation (Brunner & Bernasconi, 2005; Toyoda
251 et al., 2017). Variations in SP discriminate among nitrifier-denitrification, canonical
252 denitrification, and partial reduction sequences downstream of nitrite. Isotopomer signatures
253 must account for subsequent N_2O reduction and mixing, which may overprint primary
254 fractionation signals [\(Toyoda et al., 2017; Lewicka-Szczebak et al., 2020\)](#). ~~Direct~~
255 ~~determination of natural abundance $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in nitrite is achievable using azide-based~~
256 ~~conversion methods which reduce it to N_2O (McIlvin & Altabet, 2005; Sebilo et al., 2019),~~
257 ~~whereas analytical approaches optimized for nitrate do not independently preserve nitrite~~
258 ~~information without prior chemical conversion.~~

259 [Despite their strong mechanistic potential, nitrite isotope measurements are only feasible](#)
260 [when sufficient nitrite accumulates for analysis and remain analytically challenging. Nitrite is](#)
261 [chemically unstable and may undergo rapid oxidation or reduction during sampling and](#)
262 [storage, potentially altering its isotopic composition before analysis. Reliable preservation](#)
263 [therefore requires rapid processing and carefully controlled storage conditions, often under](#)
264 [alkaline conditions that minimize transformation rates. In many terrestrial and aquatic systems,](#)
265 [low nitrite concentrations restrict isotope analyses to zones where nitrite transiently](#)
266 [accumulates. This emphasizes the need for continued development of high-sensitivity](#)
267 [analytical approaches \(Lewicka-Szczebak et al., 2021; Deb and Lewicka-Szczebak, 2025\).](#)

268 [For instance, a recent study developed a method for freshwater samples by coupling anion-](#)
269 [exchange resin preconcentration with the azide reduction method for nitrite isotopic](#)
270 [characterization at concentrations of 0.02 \$\mu\text{mol L}^{-1}\$ or higher \(Jiang et al., 2026\). Tracer](#)
271 [additions and molecular or transcriptomic measurements characterise the potential for nitrite](#)
272 [turnover and necessarily perturb the system they interrogate. Natural-abundance isotope](#)
273 [analysis of an in situ sample is alone non-perturbative: it records the actual expression of](#)
274 [turnover under real conditions. The complementary methods therefore address a different](#)
275 [question and cannot substitute for the in situ signal. The principal analytical challenge is](#)
276 [ultimately not detection of the nitrite pool but deconvolution of a composite in situ signature](#)
277 [integrating concurrent production and consumption with their respective fractionation](#)
278 [factors.](#)~~[These analytical limitations likely contribute to the still limited application of nitrite](#)~~
279 ~~[isotope measurements in environmental studies despite their considerable interpretative](#)~~
280 ~~[potential.](#)~~

283 **Sensitivity across environmental gradients**

284 Environmental systems differ not in whether nitrite turnover operates, but in how sensitively
285 flux redistribution at the nitrite node responds to perturbation. Sensitivity is highest where
286 oxidative and reductive metabolisms overlap at [oxic-anoxic](#)~~redox~~ transition zones, and
287 attenuates where reaction coupling is constrained to a single redox regime. Small shifts in
288 oxygen supply, electron donor availability, or hydrological residence time can reorganize
289 pathway partitioning at the nitrite node without producing detectable changes in bulk nitrogen
290 pools. The nitrogen cycle is therefore most vulnerable to mechanistic misinterpretation
291 precisely where nitrite turnover is most intense.

292 In fully oxic environments, nitrite is produced during ammonia oxidation and rapidly oxidized
293 to nitrate, reflecting tight coupling between nitrification steps (Casciotti, 2016). Redox
294 transition zones — oxic–anoxic interfaces in soils, sediments, riparian zones and stratified
295 water columns — represent the most dynamic settings (Burgin et al., 2011). Simultaneous
296 production from ammonia oxidation and nitrate reduction, combined with kinetically
297 constrained consumption, promotes nitrite accumulation and amplifies sensitivity to
298 environmental change (Buchwald & Casciotti, 2010; Bristow et al., 2016). These zones are
299 recognized as hotspots of N_2O production, consistent with the central role of nitrite in
300 regulating the $\text{N}_2\text{O}:\text{N}_2$ ratio (Butterbach-Bahl et al., 2013; Babbin et al., 2020). In persistently
301 anoxic environments — deep sediments, saturated soils and oxygen minimum zones — nitrite
302 is predominantly generated via nitrate reduction and consumed through denitrification, DNRA
303 or anammox (Zumft, 1997; Lam & Kuypers, 2011; Ward et al., 2009; Dalsgaard et al., 2012;
304 Kalvelage et al., 2013; [Denk et al., 2017](#); [Deb et al., 2024](#)). Hydrological and transport

305 processes further modulate this coupling across all settings: long residence times favor
306 complete turnover, whereas rapid transport can decouple production from consumption,
307 allowing accumulation or downstream export (Sebilo et al., 2006).

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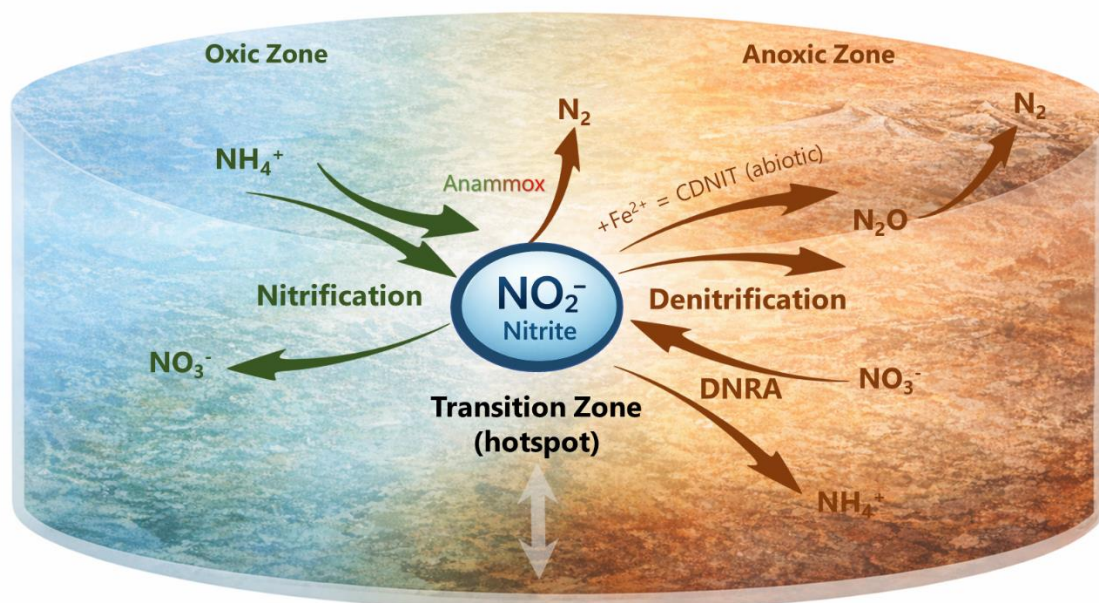
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310 **Nitrite as the control point of nitrogen fate**

311 Nitrogen cycling is commonly interpreted through the distribution of dominant inorganic pools.

312 This perspective captures accumulation and export; it does not identify where transformation
313 trajectories are decided. The reaction network places that decision at the stage of nitrite
314 turnover. Environmental conditions regulate this distribution by modulating the rates of nitrite
315 production and consumption.

316 This reframing resolves several persistent ambiguities. Similar nitrate or ammonium
317 concentrations can arise from fundamentally different internal configurations of production and
318 consumption. N₂O emissions cannot be predicted from pool size alone because their
319 magnitude depends on the completeness of reduction downstream of nitrite. Redox transition
320 zones emerge as hotspots not because they contain larger pools, but because they intensify
321 flux redistribution at this intermediate. A nitrite-centred framework shifts emphasis from
322 storage to flux (Fig. 3), from accumulation to coupling, and from static pools to dynamic
323 turnover, linking microbial metabolism, abiotic reactivity, redox heterogeneity, and
324 atmospheric exchange within a single mechanistic perspective. Because both production and
325 reduction of N₂O depend on nitrite availability and turnover, resolving dynamics at this node
326 provides a direct mechanistic link between microbial processes and climate-relevant gas
327 fluxes.



$$\text{Net} = \text{P} - \text{C}$$

328

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

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339 Changes in concentration reflects the net balance between production and consumption.
 340 $\delta^{15}\text{N}$ records the imprint of concurrent production and consumption even when pool size
 341 remains constant. Oxygen isotope exchange constrains interpretation but reinforces the
 342 diagnostic primacy of nitrogen isotopes. At the same time, the extent of oxygen exchange with
 343 water may provide information on nitrite residence times and the relative rates of biological
 344 turnover versus abiotic exchange (Buchwald and Casciotti, 2013; Lewicka-Szczebak et al.,
 345 2021). Incorporating nitrite isotopes converts an otherwise unobservable internal turnover into
 346 a measurable quantity.~~Incorporating nitrite isotopes converts an otherwise unobservable~~
 347 ~~internal turnover into a measurable quantity.~~

348 Translating this framework into practice requires prioritising direct nitrite isotope
 349 measurements alongside conventional concentration measurements, particularly in redox-
 350 dynamic systems where net fluxes are most ambiguous. Dual $\delta^{15}\text{N}$ – $\delta^{18}\text{O}$ approaches,
 351 combined with isotopomer analysis of N_2O , offer the most diagnostic power. Although nitrite
 352 isotope measurements offer strong mechanistic insight, their application is constrained by low
 353 concentrations and analytical challenges. Recent advances in high-sensitivity methods are

354 [expanding their feasibility in natural systems. Nonetheless, these limitations continue to](#)
355 [restrict their widespread use in environmental studies.](#)

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

361 As environmental systems experience increasing redox variability under climate change and
362 land-use intensification, sensitivity at the nitrite stage is likely to amplify ([Callbeck et al.,](#)
363 [20026](#)). Understanding nitrogen cycling at Earth-system scale requires resolving where and
364 how nitrite flux is redistributed. Without explicit consideration of turnover at this branching
365 node, interpretations of nitrogen budgets, greenhouse gas emissions, and isotopic signals
366 remain incomplete.

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369 **Author contribution**

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

371

372 **Competing interests**

373 The authors declare that they have no conflict of interest.

374

375 **Data and code availability**

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

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