

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 — 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 the obligatory precursor to all downstream dissolved and gaseous
24 products. Because nitrite rarely accumulates, it has often been treated as a transient
25 intermediate of limited interpretive value. This apparent invisibility reflects rapid, tightly
26 coupled turnover and does not indicate functional insignificance. Its low and frequently
27 undetectable concentration is the kinetic signature of this central position rather than evidence
28 against it: rapid coupled turnover sustains high gross flux at near-zero standing concentration.
29 Nitrogen retention, recycling and losses to the atmosphere are determined during nitrite
30 turnover, where competing pathways partition fluxes according to kinetic and environmental
31 constraints.

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

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

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44 **Introduction**

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

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

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

62 The central role of nitrite follows from its position in the reaction network. It occupies the +III
63 oxidation state, midway between ammonium and nitrate., and every transformation linking
64 oxidative and reductive branches passes through it. (Burgin & Hamilton, 2007; Lam &
65 Kuypers, 2011; Kraft et al., 2014). Nitric oxide (NO) also participates in both branches. Its half-
66 life of seconds in aqueous solution keeps its concentration below measurable levels under
67 environmental conditions, which leaves nitrite as the single dissolved intermediate accessible
68 to direct observation. No other inorganic nitrogen compound combines this dual role of
69 convergence and divergence. The fate of nitrogen at this junction is governed by the balance
70 between nitrite-producing and nitrite-consuming processes under specific redox, kinetic and
71 environmental constraints (Firestone & Davidson, 1989; Denk et al., 2017; Deb et al., 2024).

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

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

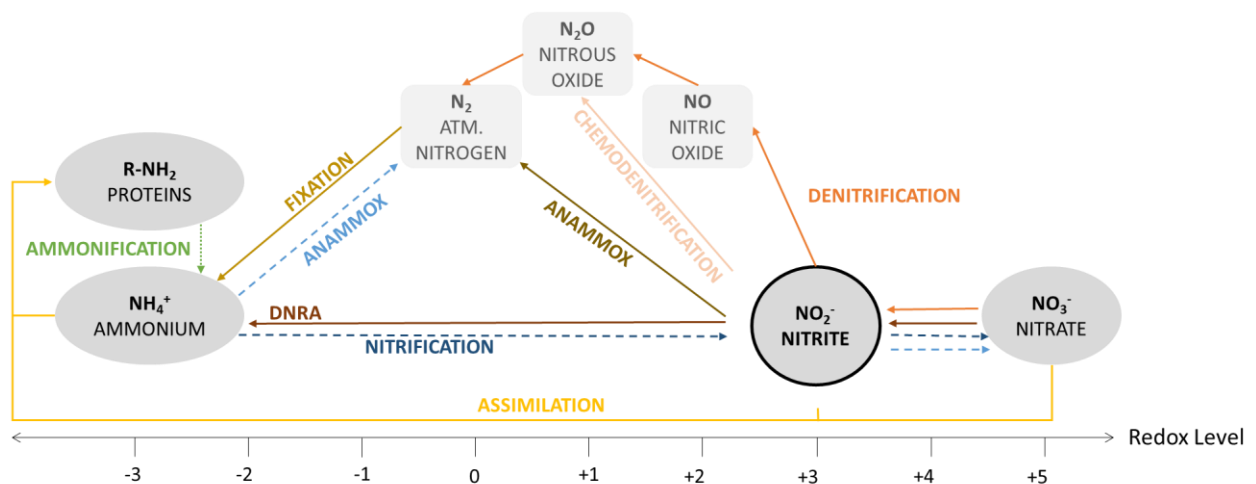
81 detail in the Isotopic constraints section below. Available methods advances permit direct
 82 determination of natural-abundance $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in nitrite (McIlvin & Altabet, 2005; Sebilo et
 83 al., 2019; Deb & Lewicka-Szczebak, 2025; Hu et al., 2026). Oxygen isotopes of nitrite are
 84 partially reset due to equilibrium with oxygen isotopes of ambient water, modifying the primary
 85 biological signal and thereby increasing the diagnostic importance of $\delta^{15}\text{N}$.
 86 Resolving nitrogen fate requires shifting analytical focus from accumulated pools to the
 87 intermediate at which pathway divergence is decided. The reaction network structure, kinetics
 88 of nitrite turnover, and stable isotope constraints together provide a mechanistic basis for
 89 predicting nitrogen budgets and N_2O emissions. This basis relies on considering nitrite as a
 90 central component of the nitrogen cycle rather than a transient residual.

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93 Nitrite as the structural branching node

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

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

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

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114 Under oxygen-limited or anoxic conditions, nitrite is produced during nitrate reduction and
115 partitioned among denitrification, DNRA, anammox, and abiotic reduction. Each pathway
116 channels nitrite toward distinct fates, from nitrogen retention to irreversible gaseous loss
117 (Zumft, 1997; Lam & Kuypers, 2011). Abiotic reactions extend this network in both redox
118 directions: reduction of nitrite by ferrous iron generates NO and N₂O independently of
119 enzymatic control, competing with biological pathways (Jones et al., 2015; Grabb et al., 2017;
120 Robinson et al., 2021), and oxidation of nitrite to nitrate by ligand-bound Mn(III) proceeds
121 abiotically under both oxic and anoxic conditions (Karolewski et al., 2021; Luther et al., 2021).
122 The defining characteristic of this convergence-divergence architecture is redistribution, not
123 accumulation. Nitrite operates as a flux junction, a node at which inputs from multiple upstream
124 pathways are portioned among competing downstream transformations, so that its
125 concentration reflects the balance between upstream formation and downstream
126 consumption, while its turnover rate determines the direction and magnitude of nitrogen
127 transfer. In spatially heterogeneous environments, nitrite produced in one microdomain may
128 be consumed in an adjacent zone within short diffusion distances (Firestone & Davidson,
129 1989). Accumulation arises primarily when this coupling is disrupted by kinetic limitation, redox
130 fluctuation, or imbalance in electron donor and acceptor supply.

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

134 If the structural position of nitrite determines where pathway divergence occurs, the kinetic
135 balance between gross production and gross consumption determines how. The direction and
136 magnitude of nitrogen redistribution at the nitrite node depend on the kinetic balance between
137 gross production and gross consumption. When these proceed at comparable rates, net
138 concentration change approaches zero while flux through the intermediate remains
139 substantial; pool size therefore provides limited information about pathway activity (Margalef-
140 Marti et al., 2026). Such tightly coupled nitrite turnover has recently been observed in nitrate-
141 rich groundwater systems using combined isotope and microbial approaches (Deb et al.,
142 2025). A distinct regime arises when consumption is limited only by the rate of supply: nitrite
143 is consumed as rapidly as it is produced, so that the standing concentration itself — not merely
144 its rate of change — approaches zero even as gross flux remains intense. This is the limiting
145 case in which an active and decisive node becomes analytically invisible.

146 Accumulation and persistence along time of nitrite is determined by the degree of kinetic
147 coupling between sequential reactions. Tight coupling in oxic systems constrains
148 accumulation and shortens residence time; disruption by fluctuating oxygen supply, transport
149 limitation, or electron donor imbalance prolongs residence time and increases the probability
150 of accumulation (see Sensitivity across environmental gradients section). Shifts in
151 environmental conditions reorganize flux distribution at the nitrite node even when nitrate or
152 ammonium pools exhibit minimal change.

153 Gaseous nitrogen (i.e., NO, N₂O and N₂) production represents a relevant downstream
154 outcome of nitrite turnover. In microbial denitrification, the relative gross rates of successive
155 reductions from nitrite to NO, N₂O, and ultimately N₂, determine the N₂O:N₂ ratio. Sustained
156 electron donor supply (such as organic matter) and active nitrous oxide reductase favour
157 completion of the sequence and N₂ dominance; partial decoupling enhances expression of
158 intermediate products, particularly N₂O (Firestone & Davidson, 1989; Zumft, 1997; Lewicka-
159 Szczebak et al., 2020). During oxygen-limited nitrification, nitrite generated from ammonium
160 oxidation may be partially reduced within ammonia oxidizers, producing N₂O under fluctuating
161 oxygen conditions (Wrage et al., 2001). Abiotic reduction of nitrite by ferrous iron and reduced
162 mineral phases generates NO and N₂O independently of enzymatic control (Jones et al., 2015;
163 Grabb et al., 2017).

164 Although N₂O is not a primary product of canonical anammox metabolism — hydrazine
165 oxidation yields N₂ directly — anammox competes with denitrifiers for the available nitrite
166 pool, thereby influencing the partitioning of nitrite between N₂-producing and N₂O-producing
167 pathways in mixed-metabolism environments (Kartal et al., 2011). Therefore, the N₂O:N₂ ratio,
168 and the relative production of N₂ versus N₂O more broadly, is controlled by turnover intensity
169 and kinetic coupling at the nitrite stage rather than by pool size.

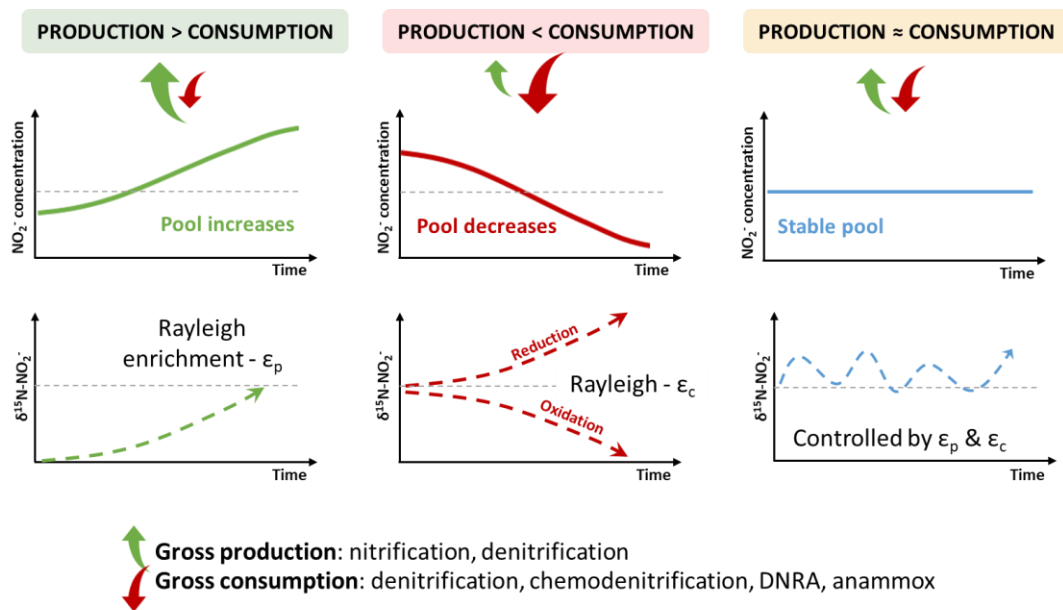
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172 **Isotopic constraints on gross nitrite turnover**

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

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181 **Figure 2. Isotopic expression of gross nitrite turnover under contrasting production-**
 182 **consumption regimes.** Top panels show nitrite concentration ([NO₂⁻]) as a function of time, reflecting
 183 the net balance between gross production (P) and gross consumption (C): accumulation when P > C,
 184 depletion when P < C, and quasi-steady state when P ≈ C despite high internal fluxes. The P ≈ C regime
 185 spans a continuum of standing concentrations: where production and consumption balance
 186 independently, nitrite stabilises at a moderate level; where consumption is limited only by the rate of
 187 supply, nitrite is consumed as fast as it forms and its concentration approaches zero despite intense
 188 gross flux. Across this continuum, δ¹⁵N(NO₂⁻) continues to evolve in response to gross turnover, so that
 189 the isotopic signal persists even where the pool itself becomes analytically invisible. Bottom panels
 190 illustrate conceptual trajectories of δ¹⁵N(NO₂⁻). In contrast to concentration, isotopic composition
 191 responds to gross turnover rather than net change. δ¹⁵N(NO₂⁻) evolves according to the isotopic
 192 signatures of contributing sources and the combined effects of isotope fractionation during production
 193 (ε_P) and consumption (ε_C). The direction of isotopic change is therefore process-dependent and not
 194 universal (e.g. inverse isotope effects during nitrite production by nitrification). Under conditions of
 195 balanced production and consumption (P ≈ C), δ¹⁵N(NO₂⁻) may drift despite constant concentration,
 196 reflecting ongoing gross turnover. Oxygen isotope signals (δ¹⁸O) may be partially overprinted by
 197 exchange with water, increasing the diagnostic primacy of δ¹⁵N.

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199 Each pathway imposes a characteristic kinetic isotope fractionation. During ammonia
 200 oxidation, inverse nitrogen isotope effects may enrich newly formed nitrite relative to its
 201 ammonium source (Casciotti et al., 2003; Santoro & Casciotti, 2011). During nitrite oxidation
 202 to nitrate, preferential removal of lighter isotopes enriches the residual pool in both ¹⁵N and
 203 ¹⁸O (Casciotti, 2009; Buchwald et al., 2012). Reductive pathways generate distinct
 204 fractionation patterns associated with nitrite reductase activity (Brunner & Bernasconi, 2005;
 205 Casciotti et al., 2010). Abiotic reduction by ferrous iron produces additional nitrogen isotope
 206 effects governed by surface-mediated electron transfer (Jones et al., 2015; Grabb et al., 2017).
 207 Abiotic oxidation of nitrite to nitrate by ligand-bound Mn(III) imparts a large inverse nitrogen
 208 isotope effect (≈ -20‰) closely resembling that of biological nitrite oxidation, with the
 209 additional oxygen atom derived from water (Karolewski et al., 2021). This convergence of
 210 biotic and abiotic fractionation factors reinforces the value of interpreting isotopic signatures

211 within environmental and redox context. Overlapping fractionation factors across biological
212 and abiotic pathways preclude simple end-member mixing and require integration with
213 environmental and redox context. The direction of $\delta^{15}\text{N}\text{-NO}_2^-$ evolution is not universal and
214 depends on the relative contributions of concurrent production and consumption pathways,
215 their associated isotope effects (ϵ_P , ϵ_C), and the isotopic composition of the source substrate.
216 Oxygen isotopes introduce an additional dimension. Exchange between nitrite oxygen atoms
217 and ambient water may proceed on timescales comparable to biological turnover (Buchwald
218 & Casciotti, 2010; Casciotti et al., 2010; Granger & Wankel, 2016), attenuating the
219 independence of $\delta^{18}\text{O}$ as a pathway tracer. Nitrogen isotopes do not undergo analogous
220 exchange, preserving sensitivity to pathway-specific fractionation. Under conditions of
221 significant oxygen isotope exchange, $\delta^{15}\text{N}$ provides the more robust constraint on gross
222 turnover.

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

232 Despite their strong mechanistic potential, nitrite isotope measurements are only feasible
233 when sufficient nitrite accumulates for analysis and remain analytically challenging. Nitrite is
234 chemically unstable and may undergo rapid oxidation or reduction during sampling and
235 storage, potentially altering its isotopic composition before analysis. Reliable preservation
236 therefore requires rapid processing and carefully controlled storage conditions, often under
237 alkaline conditions that minimize transformation rates. In many terrestrial and aquatic systems,
238 low nitrite concentrations restrict isotope analyses to zones where nitrite transiently
239 accumulates. This emphasizes the need for continued development of high-sensitivity
240 analytical approaches (Lewicka-Szczebak et al., 2021; Deb and Lewicka-Szczebak, 2025).
241 For instance, a recent study developed a method for freshwater samples by coupling anion-
242 exchange resin preconcentration with the azide reduction method for nitrite isotopic
243 characterization at concentrations of $0.02 \mu\text{mol L}^{-1}$ or higher (Jiang et al., 2026). Tracer
244 additions and molecular or transcriptomic measurements characterise the potential for nitrite
245 turnover and necessarily perturb the system they interrogate. Natural-abundance isotope
246 analysis of an in situ sample is alone non-perturbative: it records the actual expression of
247 turnover under real conditions. The complementary methods therefore address a different

248 question and cannot substitute for the in situ signal. The principal analytical challenge is
249 ultimately not detection of the nitrite pool but deconvolution of a composite in situ signature
250 integrating concurrent production and consumption with their respective fractionation factors.

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253 **Sensitivity across environmental gradients**

254 Environmental systems differ not in whether nitrite turnover operates, but in how sensitively
255 flux redistribution at the nitrite node responds to perturbation. Sensitivity is highest where
256 oxidative and reductive metabolisms overlap at oxic-anoxic transition zones, and attenuates
257 where reaction coupling is constrained to a single redox regime. Small shifts in oxygen supply,
258 electron donor availability, or hydrological residence time can reorganize pathway partitioning
259 at the nitrite node without producing detectable changes in bulk nitrogen pools. The nitrogen
260 cycle is therefore most vulnerable to mechanistic misinterpretation precisely where nitrite
261 turnover is most intense.

262 In fully oxic environments, nitrite is produced during ammonia oxidation and rapidly oxidized
263 to nitrate, reflecting tight coupling between nitrification steps (Casciotti, 2016). Redox
264 transition zones — oxic–anoxic interfaces in soils, sediments, riparian zones and stratified
265 water columns — represent the most dynamic settings (Burgin et al., 2011). Simultaneous
266 production from ammonia oxidation and nitrate reduction, combined with kinetically
267 constrained consumption, promotes nitrite accumulation and amplifies sensitivity to
268 environmental change (Buchwald & Casciotti, 2010; Bristow et al., 2016). These zones are
269 recognized as hotspots of N₂O production, consistent with the central role of nitrite in
270 regulating the N₂O:N₂ ratio (Butterbach-Bahl et al., 2013; Babbin et al., 2020). In persistently
271 anoxic environments — deep sediments, saturated soils and oxygen minimum zones — nitrite
272 is predominantly generated via nitrate reduction and consumed through denitrification, DNRA
273 or anammox (Zumft, 1997; Lam & Kuypers, 2011; Ward et al., 2009; Dalsgaard et al., 2012;
274 Kalvelage et al., 2013; Denk et al., 2017; Deb et al., 2024). Hydrological and transport
275 processes further modulate this coupling across all settings: long residence times favor
276 complete turnover, whereas rapid transport can decouple production from consumption,
277 allowing accumulation or downstream export (Sebilo et al., 2006).

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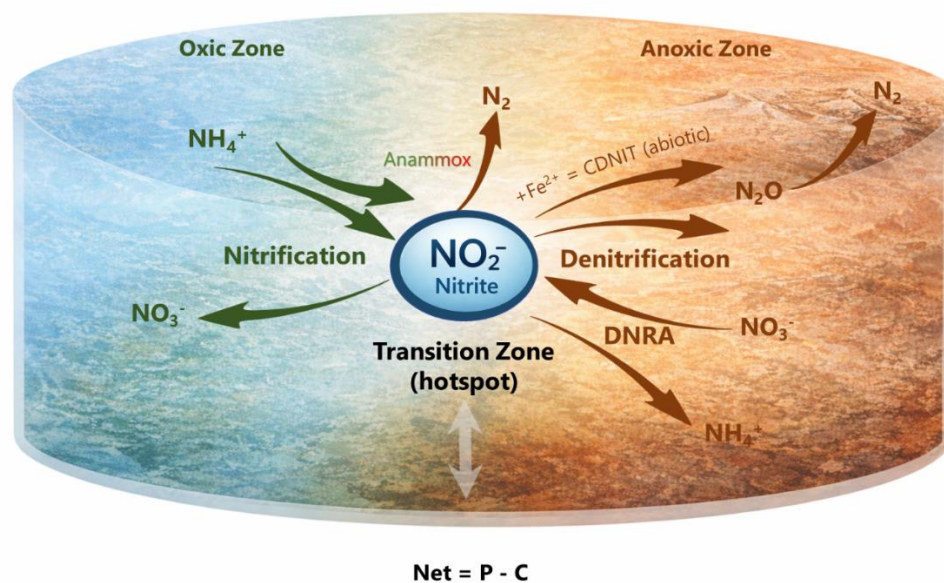
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280 **Nitrite as the control point of nitrogen fate**

281 Nitrogen cycling is commonly interpreted through the distribution of dominant inorganic pools.
282 This perspective captures accumulation and export, it does not identify where transformation
283 trajectories are decided. The reaction network places that decision at the stage of nitrite

284 turnover. Environmental conditions regulate this distribution by modulating the rates of nitrite
285 production and consumption.

286 This reframing resolves several persistent ambiguities. Similar nitrate or ammonium
287 concentrations can arise from fundamentally different internal configurations of production and
288 consumption. N_2O emissions cannot be predicted from pool size alone because their
289 magnitude depends on the completeness of reduction downstream of nitrite. Redox transition
290 zones emerge as hotspots not because they contain larger pools, but because they intensify
291 flux redistribution at this intermediate. A nitrite-centred framework shifts emphasis from
292 storage to flux (Fig. 3), from accumulation to coupling, and from static pools to dynamic
293 turnover, linking microbial metabolism, abiotic reactivity, redox heterogeneity, and
294 atmospheric exchange within a single mechanistic perspective. Because both production and
295 reduction of N_2O depend on nitrite availability and turnover, resolving dynamics at this node
296 provides a direct mechanistic link between microbial processes and climate-relevant gas
297 fluxes.



298

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

307

308 Changes in concentration reflect the net balance between production and consumption. $\delta^{15}N$
309 records the imprint of concurrent production and consumption even when pool size remains
310 constant. Oxygen isotope exchange constrains interpretation but reinforces the diagnostic

311 primacy of nitrogen isotopes. At the same time, the extent of oxygen exchange with water may
312 provide information on nitrite residence times and the relative rates of biological turnover
313 versus abiotic exchange (Buchwald and Casciotti, 2013; Lewicka-Szczebak et al., 2021).
314 Incorporating nitrite isotopes converts an otherwise unobservable internal turnover into a
315 measurable quantity.

316 Translating this framework into practice requires prioritising direct nitrite isotope
317 measurements alongside conventional concentration measurements, particularly in redox-
318 dynamic systems where net fluxes are most ambiguous. Dual $\delta^{15}\text{N}$ – $\delta^{18}\text{O}$ approaches,
319 combined with isotopomer analysis of N_2O , offer the most diagnostic power. Although nitrite
320 isotope measurements offer strong mechanistic insight, their application is constrained by low
321 concentrations and analytical challenges. Recent advances in high-sensitivity methods are
322 expanding their feasibility in natural systems. Nonetheless, these limitations continue to
323 restrict their widespread use in environmental studies.

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

329 As environmental systems experience increasing redox variability under climate change and
330 land-use intensification, sensitivity at the nitrite stage is likely to amplify (Callbeck et al.,
331 20026). Understanding nitrogen cycling at Earth-system scale requires resolving where and
332 how nitrite flux is redistributed. Without explicit consideration of turnover at this branching
333 node, interpretations of nitrogen budgets, greenhouse gas emissions, and isotopic signals
334 remain incomplete.

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337 **Author contribution**

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

339

340 **Competing interests**

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

342

343 **Data and code availability**

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

346

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