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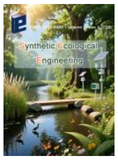

Review Article

Microbial Nitrogen Transformation in Hyporheic–riparian Zones: Integrating Molecular Ecology and Biogeochemical Perspectives

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KEYWORDS
hyporheic–riparian zone
nitrogen cycling
denitrification
anammox
microbial functional ecology
ecohydrology; metagenomics

ABSTRACT

Hyporheic–riparian zones (HRZs) function as critical biogeochemical hotspots, where dynamic exchanges between surface water and groundwater establish steep physicochemical gradients that regulate nitrogen (N) cycling at the terrestrial–aquatic interface. Here, we synthesises advances in the understanding of microbial N transformation pathways within HRZs from integrated biogeochemical, hydrological, and molecular ecological perspectives. We critically evaluated major microbially mediated processes, including nitrification, comammox, denitrification, dissimilatory nitrate reduction to ammonium (DNRA), anaerobic ammonium oxidation (anammox), and iron-coupled ammonium oxidation (feammox), in relation to redox stratification, hydrological residence time, organic carbon availability, and sediment heterogeneity. Emphasis is placed on emerging evidence for coupled anaerobic N-loss pathways and their sensitivity to transient hydrological exchange. Recent applications of metagenomics, stable isotope probing, and microbial network analysis have substantially advanced the mechanistic understanding of functional diversity and pathway interactions governing ecosystem N fate. We further assessed how anthropogenic pressures eutrophication, flow regulation, and climate-driven hydrological variability alter microbial community structure and N-cycling efficiency. Despite considerable progress, critical knowledge gaps persist in linking ecohydrological dynamics with microbial functional ecology at spatiotemporal scales. Future research integrating multi-omics, high-resolution isotope tracing, and predictive modelling frameworks is essential for advancing mechanistic understanding and informing the sustainable management of N cycling in HRZ ecosystems.

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ARTICLE INFO

Received: 6 January 2026 | Revised: 26 May 2026 | Accepted: 27 May 2026 | Published Online: 28 May 2026

DOI: https://doi.org/10.65773/see.2.1.103

1. Introduction

Nitrogen (N) is a fundamental element in Earth's biogeochemical systems, exerting critical control over ecosystem productivity, microbial metabolism, and nutrient dynamics across terrestrial and aquatic environments. As an essential macronutrient, nitrogen availability governs primary production, biodiversity maintenance, and ecosystem stability [1]. Although molecular dinitrogen (N_2) constitutes approximately 78% of the atmosphere, it must be converted into reactive forms before becoming biologically accessible. This conversion is mediated through a complex suite of microbially driven processes collectively termed the nitrogen cycle including nitrogen fixation, nitrification, denitrification, anaerobic ammonium oxidation (anammox), dissimilatory nitrate reduction to ammonium (DNRA), and organic nitrogen mineralization [2].

Over the past century, anthropogenic perturbations including industrial fertilizer synthesis, fossil fuel combustion, wastewater discharge, intensive agriculture, and land-use transformation have profoundly altered global nitrogen cycling [3]. Elevated reactive nitrogen loading to aquatic ecosystems has accelerated eutrophication, promoted hypoxia, driven biodiversity decline, contaminated groundwater reserves, and enhanced greenhouse gas emissions, collectively threatening freshwater ecosystem integrity and water resource sustainability. Consequently, elucidating the environmental controls and microbial mechanisms governing nitrogen transformation within natural attenuation systems has emerged as a critical research priority.

HRZs represent highly dynamic ecological interfaces that integrate surface water, groundwater, riparian soils, and streambed sediments. These environments are increasingly recognized as biogeochemical hotspots that regulate nutrient retention, transformation, and export at the terrestrial–aquatic interface [4,5]. In contrast to adjacent stream channels, riparian soils, or deeper aquifer systems, HRZs are distinguished by vigorous hydrological exchange that generates steep physicochemical and redox gradients over fine spatial and temporal scales (Figure 1). Such gradients establish closely coupled aerobic and anaerobic microhabitats supporting metabolically diverse microbial communities and multiple concurrent nitrogen transformation pathways [1,2]. Accordingly, HRZs function as highly reactive ecological filters that modulate nitrogen retention, permanent removal, and downstream solute transport across catchment systems.

The hydro-biogeochemical heterogeneity inherent to HRZs fundamentally shapes microbial community composition, functional gene distribution, and pathway dominance. Oxic microzones proximal to sediment–water interfaces typically support nitrification and aerobic ammonia oxidation, whereas adjacent suboxic and anoxic zones favor denitrification, DNRA, anammox, and emerging pathways including sulfate-dependent ammonium oxidation (sulfammox) and iron-coupled ammonium oxidation (feammox) [4,5]. This fine-scale spatial coupling of contrasting redox processes distinguishes HRZs from surrounding environments where nitrogen cycling is typically less dynamic and more geochemically constrained. Consequently, HRZs represent critical control points for watershed nitrogen budgets, nitrous oxide (N_2O) production, and downstream water quality [6].

Recent advances in molecular ecology and environmental microbiology have substantially enhanced mechanistic understanding of nitrogen cycling within HRZs [6]. High-throughput sequencing, metagenomics, metatranscriptomics, environmental DNA (eDNA) analysis, quantitative PCR (qPCR), and stable isotope probing (SIP) now permit direct linkage between microbial identity, functional gene expression, and nitrogen transformation activity under field-relevant conditions [7]. These methodological advances have revealed previously unrecognized microbial taxa, cryptic metabolic pathways, and pronounced spatial heterogeneity in nitrogen cycling tightly associated with hydrological exchange dynamics and redox variability [7,8]. Emerging discoveries—including complete ammonia oxidation (comammox) [9], nitrate/nitrite-dependent anaerobic methane oxidation (N-DAMO), sulfammox, and feammox [10] further underscore the metabolic complexity of nitrogen turnover in HRZ ecosystems.

Despite considerable progress, substantial knowledge gaps persist regarding the mechanisms governing pathway dominance, inter-guild microbial interactions, and ecosystem-scale nitrogen flux regulation in HRZs. In particular, the integration of hydrological dynamics, redox variability, microbial functional ecology, and high-resolution molecular datasets into predictive ecosystem frameworks remains insufficiently developed [11,12]. Moreover, existing reviews frequently address nitrogen cycling pathways or molecular tools in isolation, with limited attention to how hydrological exchange structures microbial communities and regulates the coupled nitrogen transformations unique to HRZ environments.

This review provides an integrated synthesis of nitrogen cycling in HRZs by linking hydrological processes, environmental gradients, microbial functional guilds, and emerging molecular approaches within a unified conceptual framework. Specifically, this review: (i) examines the major microbially mediated nitrogen transformation pathways operating in HRZs; (ii) evaluates how hydrological exchange, redox gradients, and geochemical conditions regulate microbial community structure and inter-pathway interactions; (iii) critically assesses recent molecular and isotopic methodologies applied to investigate HRZ nitrogen dynamics; and (iv) identifies emerging pathways, unresolved questions, and future research directions necessary for improving ecosystem-scale predictions of nitrogen fluxes. By integrating microbial ecology, hydrology, and biogeochemistry, this review aims to advance mechanistic understanding of nitrogen transformation processes in HRZs and inform the development of sustainable watershed management strategies under intensifying anthropogenic and climatic pressures.

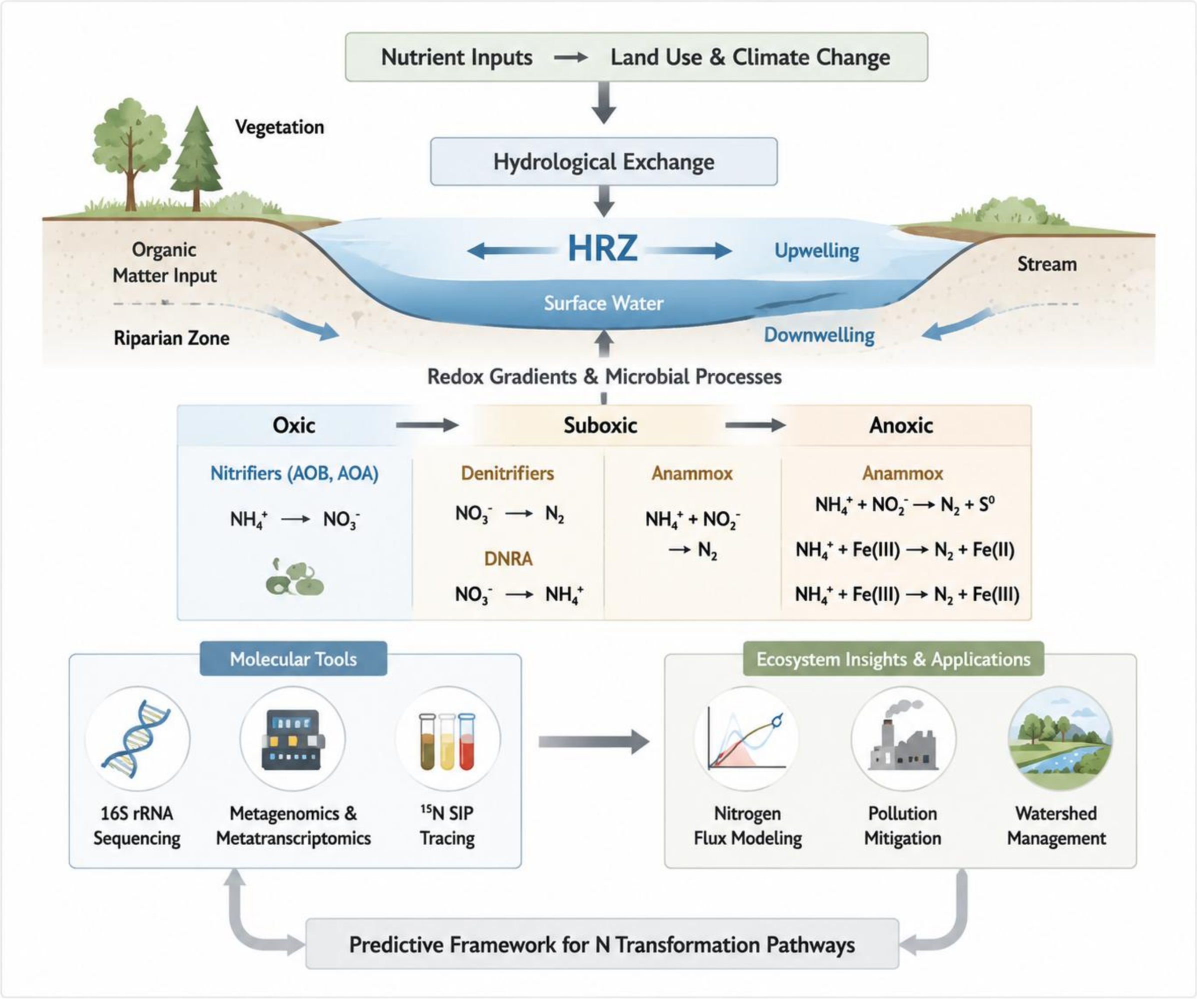

Figure 1. Nitrogen transformation pathways in the hyporheic-reparian zone: This schematic illustrates nitrogen cycling within the hyporheic zone (HRZ) of stream ecosystems, highlighting hydrological exchange, redox gradients, and microbial processes across oxic, suboxic, and anoxic zones. Molecular tools, including 16S rRNA sequencing, metagenomics, and stable isotope tracing, inform predictive frameworks that enable ecosystem-level insights such as nitrogen flux modeling, pollution mitigation, and watershed management. The design emphasizes clarity and professional visualization suitable for academic presentations.

2. Concept of hyporheic–riparian zones (HRZs)

Hyporheic–riparian zones constitute dynamic ecological interfaces situated at the convergence of surface water, groundwater, streambed sediments, and adjacent terrestrial environments. The hyporheic zone denotes the saturated subsurface region beneath and lateral to streambeds where active bidirectional mixing of stream water and groundwater occurs, whereas the riparian zone encompasses the vegetated terrestrial corridor flanking rivers and streams [8-10]. Collectively, these hydrologically interconnected zones regulate solute exchange, nutrient retention, contaminant attenuation, and microbially mediated biogeochemical transformations, thereby exerting disproportionate influence on watershed-scale ecosystem functioning [12].

In contrast to stream channels or groundwater systems considered independently, HRZs are distinguished by steep physicochemical gradients generated through continuous surface–subsurface hydrological exchange [3]. Spatial and temporal variations in dissolved oxygen concentration, redox potential, hydraulic residence time, organic carbon availability, and nutrient loading create a mosaic of heterogeneous microbial habitats that support metabolically diverse nitrogen transformation pathways [4,5]. These conditions establish HRZs as biogeochemical hotspots where coupled aerobic and anaerobic processes including nitrification, denitrification, dissimilatory nitrate reduction to ammonium (DNRA), and anaerobic ammonium oxidation (anammox) occur simultaneously over fine spatial scales [6,7].

Recent hydrological investigations demonstrate that HRZs substantially influence nitrogen retention and permanent removal despite occupying relatively small spatial areas within catchments [11]. Their ecological significance derives from rapid solute turnover, transient storage dynamics, and metabolically active microbial biofilms that mediate nitrogen transformations under fluctuating environmental conditions. In particular, the interaction between hydrological flowpaths and sediment heterogeneity promotes intimate spatial coupling of oxidative and reductive nitrogen processes, enabling HRZs to function as effective natural bioreactors for nitrogen attenuation [10,11]. The concept of "hot spots and hot moments" has been widely applied to characterize the spatiotemporal variability of nitrogen removal intensity within these zones, where disproportionately high reaction rates coincide with specific hydrological and geochemical conditions [6].

The spatial boundaries of HRZs remain inherently difficult to delineate because hydrological connectivity varies temporally with stream discharge, groundwater table fluctuations, and seasonal climatic forcing [12]. Contemporary conceptualizations therefore define HRZs functionally rather than strictly geographically, emphasizing residence time distributions, exchange intensity, sediment permeability, and ecological activity [3,12]. This process-based perspective has substantially advanced understanding of how HRZ spatial extent and biogeochemical reactivity respond dynamically to changing hydrological regimes [13].

Advances in ecohydrology and molecular microbial ecology have fundamentally transformed HRZ research over the past decade. Integrated approaches combining reactive transport modeling, conservative and reactive hydrological tracers, high-resolution dissolved oxygen profiling, metagenomics, metatranscriptomics, and compound-specific stable isotope techniques now provide mechanistic insight into the causal linkages between hydrological exchange dynamics, microbial functional diversity, and nitrogen transformation rates [13,14]. These methodological developments have shifted HRZ research from descriptive characterization toward predictive frameworks capable of identifying nitrogen retention hotspots and forecasting ecosystem responses to anthropogenic disturbance, flow regulation, and climate variability.

2.1 Biogeochemistry of the nitrogen cycle in HRZs

Nitrogen cycling within HRZs is controlled by the interaction of hydrological exchange, sediment geochemistry, microbial functional diversity, and redox heterogeneity. Surface water infiltration and groundwater upwelling generate steep oxic–anoxic gradients that regulate the distribution and activity of nitrogen-transforming microorganisms [4]. These gradients produce a mosaic of microenvironments that support tightly coupled nitrogen transformation pathways, often occurring simultaneously over centimeter-scale distances.

The principal nitrogen transformation processes occurring in HRZs include nitrogen fixation, ammonification, nitrification, denitrification, DNRA, and anaerobic ammonium oxidation (anammox). Emerging pathways such as complete ammonia oxidation (comammox), nitrate/nitrite-dependent anaerobic methane oxidation (N-DAMO), sulfammox, and feammox further expand the recognized complexity of nitrogen cycling in these environments

[15,16]. Collectively, these pathways regulate the conversion of nitrogen between reduced, oxidized, and gaseous forms, thereby controlling nitrogen retention, recycling, and permanent removal.

Microbial functional guilds drive these transformations and respond rapidly to environmental fluctuations. Ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and nitrite-oxidizing bacteria (NOB) dominate oxygenated sediments and regulate nitrification, while facultative anaerobic denitrifiers such as *Pseudomonas*, *Paracoccus*, and *Thiobacillus* thrive within suboxic zones [17]. Anammox bacteria, including *Candidatus Brocadia*, contribute substantially to anaerobic nitrogen removal in reduced sediments, whereas DNRA -associated taxa such as *Shewanella* and *Desulfovibrio* promote nitrogen retention through nitrate reduction to ammonium [18].

The balance among these pathways is strongly controlled by hydrological residence time, redox oscillations, carbon availability, and nutrient loading as conceptual frameworks of nitrogen cycling in hyporheic–riparian systems (Figure 2). Oxygenated flow paths near sediment–water interfaces favor nitrification and comammox, whereas deeper reduced sediments support denitrification, anammox, sulfammox, and feammox. Fine-grained sediments with high organic carbon availability commonly promote DNRA dominance, while fluctuating redox conditions enable rapid switching between nitrogen retention and nitrogen loss pathways [19,20]. These interactions distinguish HRZs from adjacent stream channels and aquifers, where environmental gradients are comparatively less dynamic.

Recent advances in stable isotope tracing, metagenomics, metatranscriptomics, and environmental metabolomics have substantially improved understanding of HRZ nitrogen biogeochemistry [21,22]. Combined molecular–isotopic approaches now enable direct linkage of microbial community structure and functional genes to in situ nitrogen transformation rates. Integrating these datasets with reactive transport models has advanced the development of predictive frameworks capable of forecasting nitrogen fluxes under varying hydrological and climatic conditions.

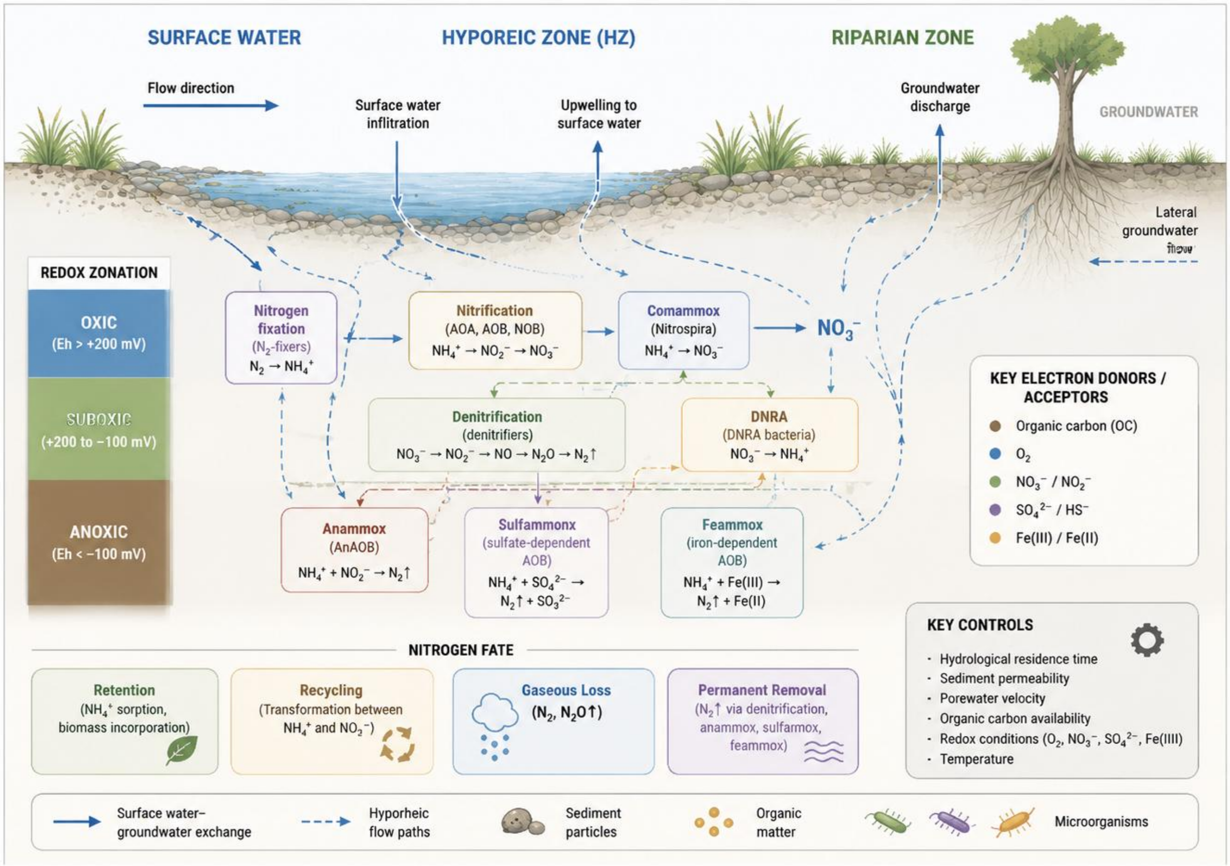

Figure 2. Conceptual framework of nitrogen cycling across stream-riparian interfaces: This figure depicts the conceptual model of nitrogen transformations within surface water, hyporheic, and riparian zones. It highlights redox zonation (oxic, suboxic, anoxic), key microbial processes such as nitrogen fixation, nitrification, comammox, denitrification, DNRA, anammox, sulfammox, and feammox, and their influence on nitrogen fate outcomes including retention, recycling, gaseous loss, and permanent removal. Surface water–groundwater exchanges and hyporheic flow paths are illustrated with arrows, while key electron donors, acceptors, and environmental controls are indicated to emphasize factors regulating nitrogen cycling in stream-riparian ecosystems.

3. Microbial nitrogen transformation processes in HRZs

Nitrogen cycling in HRZs is governed by a tightly coupled network of microbial processes that operate across steep redox and hydrological gradients. Unlike homogeneous aquatic or terrestrial systems, HRZs function as spatially structured reaction domains where nitrogen transformation pathways occur simultaneously and compete for shared substrates such as ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) [12]. The net nitrogen outcome—retention or removal—is therefore determined by process interaction strength rather than the presence of specific microbial taxa alone [3,4,24].

At the system level, HRZ nitrogen cycling can be conceptualized as three interdependent functional regimes: (i) oxidative production, (ii) reductive removal, and (iii) conservative recycling. Oxidative zones are dominated by nitrification and comammox, where ammonia is converted to nitrate under oxygenated conditions, often near sediment–water interfaces [23,43]. Reductive zones are characterized by denitrification and anammox, which mediate permanent nitrogen loss as N_2 and N_2O under low-oxygen conditions [15,28]. Conservative recycling zones are defined by DNRA activity, which retains nitrogen in bioavailable ammonium form, thereby reducing net ecosystem nitrogen export [26,39].

3.1. Spatial coupling of functional regimes

A defining feature of HRZs is the spatial coupling of these regimes over centimeter-scale redox gradients, which enables rapid substrate exchange between aerobic and anaerobic microbial communities. Nitrification produces NO_2^- and NO_3^- that are immediately consumed by downstream denitrifiers, DNRA bacteria, or anammox organisms depending on local electron donor availability and hydrological residence time [18,24]. This creates a dynamic internal nitrogen loop where production and consumption processes are tightly interdependent rather than sequential. As demonstrated in hyporheic sediment column experiments, water chemistry shifts and variable mixing regimes rapidly alter microbial community structure and corresponding denitrification, DNRA, and nitrification rates, indicating that these functional regimes are kinetically responsive to physical forcing on timescales of hours to days.

Concurrent transcriptional activity for nitrification, denitrification, nitrite-dependent anaerobic methane oxidation (N-DAMO), and anammox has been documented in both oxic and dysoxic groundwater environments, providing direct evidence that oxic–anoxic interfaces within HRZs sustain simultaneous operation of ostensibly incompatible metabolic pathways. This co-occurrence is facilitated by microscale oxygen heterogeneity rather than strict zonation, with potential synergisms through metabolite exchange (e.g., nitrite and oxygen) across redox interfaces.

3.2. Denitrification and anammox: principal nitrogen loss pathways

Denitrification and anammox represent the principal nitrogen loss pathways in HRZs. Denitrification is typically favored under high organic carbon availability and fluctuating redox conditions, while anammox dominates in more stable anoxic microsites where nitrite supply is sustained through coupled nitrification or partial denitrification activity [2,28]. In pristine carbonate-rock aquifers, for example, chemolithoautotrophic anammox has been identified as the primary driver of nitrogen loss, highlighting that heterotrophic denitrification is not universally dominant in subsurface environments. Together, these pathways regulate gaseous nitrogen emissions and constitute the primary mechanism for permanent nitrogen removal in HRZ environments [15,18].

The relative partitioning between denitrification and anammox is further modulated by human activity intensity. In subtropical estuarine systems, anthropogenic nutrient loading shifts the balance toward denitrification dominance by increasing organic carbon and nitrate availability, whereas anammox maintains a higher proportional contribution in less disturbed settings. This observation has important implications for predicting nitrogen removal efficiency along land-use gradients in HRZ-connected watersheds [38].

Fragmented denitrification pathway encoding is widespread among groundwater bacteria, although complete denitrifiers drive a considerable proportion of associated transcriptional activity, especially under dysoxic conditions [1]. Notably, most N_2O reductase genes and transcripts in these systems are of the atypical Sec-dependent type, suggesting energy-efficiency prioritization in subsurface nitrogen cycling [1]. This metabolic fragmentation implies that nitrogen loss via denitrification is frequently a community-level process requiring cooperative interactions among multiple taxa, rather than a pathway completed within single organisms.

3.3. Dissimilatory nitrate reduction to ammonium (DNRA): The Nitrogen Retention Counterbalance

DNRA functions as a nitrogen retention pathway, particularly under high carbon-to-nitrate ratios and strongly reducing conditions. By reducing NO_3^- to NH_4^+, DNRA competes directly with denitrification for nitrate, thereby controlling whether nitrogen is conserved within sediments or lost to the atmosphere [26,39]. The competitive outcome between these two pathways has been extensively investigated in both chemostat enrichment cultures and field settings. Under nitrate-limited conditions, DNRA holds a thermodynamic and kinetic competitive advantage over denitrification, while the acetate-to-nitrate ratio (Ac/N) modulates pathway dominance across a broad range of environmental conditions [26].

Nitrite also plays a regulatory role in DNRA–denitrification competition. When nitrite replaces nitrate as the terminal electron acceptor, competitive dynamics shift, indicating that the form of oxidized nitrogen substrate—not merely its concentration—shapes pathway partitioning. Resource-ratio theory has been applied to formalize these competitive interactions, demonstrating that dual-substrate limitation (organic carbon and nitrate) determines which pathway dominates under given stoichiometric conditions [2]. In hyporheic zone sediments specifically, the competition between heterotrophic denitrification and DNRA has been shown to significantly influence nitrate fate along flow paths [45].

Recent modeling approaches integrating reactive transport with microbial kinetics have begun to predict competitive outcomes in partially saturated soil–water systems, revealing that environmental modulation of DNRA versus denitrification is spatially explicit and sensitive to local moisture and redox conditions. The balance between these two pathways is therefore a critical determinant of HRZ nitrogen retention capacity [24], with implications for downstream water quality and ecosystem nitrogen budgets.

3.4. Complete ammonia oxidation (comammox) and emerging hybrid pathways

Emerging microbial processes further expand the functional diversity of HRZ nitrogen cycling. Comammox organisms (*Nitrospira* spp.) perform complete oxidation of ammonia to nitrate within a single organism, eliminating the spatial separation between ammonia-oxidizing and nitrite-oxidizing steps that characterizes canonical two-step nitrification [43]. Physiological data indicate that comammox *Nitrospira* functionally outcompete other canonical nitrifiers under highly oligotrophic conditions due to superior ammonia oxidation kinetics and metabolic versatility [3]. These organisms are environmentally widespread and numerically abundant in both natural and engineered habitats; quantitative PCR analyses across 300 Chinese forest soils demonstrate that comammox clade A and clade B co-occur with AOA, AOB, and NOB, with relative abundances modulated by soil pH [3]. In eutrophic lake sediments, comammox diversity and abundance are further influenced by nutrient loading, with sewage discharge altering community composition [16]. This broad distribution pattern suggests that comammox may contribute substantially to nitrification in oxygen-limited HRZ sediments where ammonia concentrations are low—conditions that disfavor canonical AOB but favor the high-affinity kinetics of comammox organisms.

Similarly, nitrite/nitrate-dependent anaerobic methane oxidation (N-DAMO) links methane oxidation to denitrification under anoxic conditions, coupling carbon and nitrogen cycles and contributing to nitrogen loss in methane-rich HRZ environments [18]. The integration of N-DAMO with anammox in engineered systems demonstrates that these processes can co-exist and achieve simultaneous nitrogen and methane removal under controlled nitrogen loadings, with phylogenetic and metabolic diversity of the associated microbial guilds responding to operational conditions. These processes indicate that HRZ nitrogen cycling is not limited to classical pathways but includes hybrid metabolic networks that integrate multiple biogeochemical cycles.

3.5. Alternative pathways: sulfammox, feammox, and coupled element cycling

Recent studies highlight alternative nitrogen transformation pathways such as sulfate reducing ammonium oxidation (sulfammox) and ferric iron-reducing ammonium oxidation (feammox), which couple nitrogen loss to sulfur and iron redox cycling, respectively. These pathways (Table 1) are particularly relevant in HRZs where fluctuating redox conditions and mineral heterogeneity provide diverse electron acceptors [29,32,34]. In the hyporheic zone of effluent-dominated rivers, redox gradient zonation has been shown to drive coupled nitrogen–sulfur cycling, with sulfur-oxidizing and sulfate-reducing bacteria mediating nitrate reduction through chemolithoautotrophic pathways linked to pyrite oxidation and sulfide availability. The phosphorus level gradient additionally modulates nitrogen cycling in wetland ecosystems by influencing microbial C–N–P coupling, creating interactive effects between nutrient stoichiometry and pathway partitioning [42].

Although the quantitative contribution of sulfammox and feammox to total nitrogen removal in HRZs remains uncertain, their presence underscores the expanded biochemical flexibility of nitrogen cycling at sedimentary interfaces. Tidal dynamics in intertidal flats further illustrate how physical forcing generates geochemical gradients that regulate potential coupling among carbon-, nitrogen-, and sulfur-cycling microbes along vertical redox gradients [22]. These observations collectively support the conceptualization of HRZ nitrogen cycling as a multi-element, spatially structured process whose diversity far exceeds classical representations based on nitrification–denitrification alone.

Table 1. Principal microbial nitrogen transformation pathways in HRZs and their functional attributes.

Pathway	Net N Outcome	Key Substrates	Redox Requirement	Representative Taxa	Ecological Role in HRZs	Reference
Nitrification (canonical)	$NH_4^+ \rightarrow NO_3^-$	NH_4^+, O_2	Aerobic	AOA, AOB, NOB	Oxidative N production; substrate supply	[45,40]
Comammox	$NH_4^+ \rightarrow NO_3^-$	NH_4^+, O_2	Micro-aerobic to aerobic	*Nitrospira* spp.	Efficient nitrification under oligotrophic conditions	[16,43[61]
Denitrification	$NO_3^- \rightarrow N_2$	NO_3^-, organic C	Anoxic–suboxic	*Pseudomonas*, *Paracoccus*, *Thiobacillus*	Permanent N removal	[25,53]
Anammox	$NH_4^+ + NO_2^- \rightarrow N$	NH_4^+, NO_2^-	Strictly anoxic	*Ca. Brocadia, Ca. Kuenenia*	Autotrophic N removal; low-C environments	[17,28,48]
DNRA	$NO_3^- \rightarrow NH_4^+$	NO_3^-, organic C / S^{2-}	Anoxic–reducing	*Shewanella*, *Desulfovibrio*	N retention; counterbalances denitrification	[2,26,32]
N-DAMO	$NO_2^-/NO_3^- + CH$	CH_4, NO_x	Anoxic	*Ca. Methylomirabilis, Ca. Methanoperedens*	Coupled C–N removal	[18,62]
Sulfammox / Feammox	$NH_4^+ \rightarrow N_2$ (coupled to S/Fe)	NH_4^+, Fe^{3+} / SO_4^{2-}	Variable redox	Diverse consortia	Alternative N loss under mineral-rich conditions	[30-32]

3.6. Microbial interaction networks and process coupling

Microbial nitrogen cycling in HRZs is sustained by metabolically interconnected microbial networks in which functional groups interact through substrate exchange, competition, and syntrophy. These interactions enhance system resilience while simultaneously regulating nitrogen retention and loss outcomes [41,47]. The spatial architecture of these networks is fundamentally shaped by the steep physicochemical gradient characteristic of hyporheic–riparian interfaces, where millimeter-to-centimeter scale transitions in redox potential create juxtaposed aerobic and anaerobic microhabitats that sustain metabolically incompatible processes in close proximity [12].

A key feature of HRZ microbial ecology is sequential metabolic coupling, where products of one process serve as substrates for another. For example, ammonium generated via mineralization and DNRA fuels nitrification, while nitrification-derived nitrate supports denitrification and anammox in adjacent anoxic zones [23,26]. This creates vertically and laterally stratified nitrogen transformation chains that are tightly constrained by redox gradients. In riparian zone sediments of the Wei River, ^{15}N isotope tracing revealed that denitrification, DNRA, and anammox jointly achieved natural nitrogen attenuation at rates of 4.14–35.19 $\mu mol \cdot N \cdot kg^{-1} \cdot h^{-1}$, with denitrification accounting for 59.6% of total removal, demonstrating the quantitative importance of coupled pathway interactions [3].

Competitive interactions are equally important in determining nitrogen fate. DNRA and denitrification compete for nitrate under anoxic conditions, with DNRA favored under high organic carbon availability and strong reducing environments, leading to nitrogen retention rather than removal [26,39]. Chemostat enrichment experiments demonstrate that these two pathways coexist over a broad range of acetate-to-nitrate ratios, with the competitive outcome governed by dual-substrate limitation rather than simple exclusion [48]. In hyporheic zone sediments specifically, reactive transport modeling coupled with microbial kinetics reveals that the denitrification–DNRA competition significantly controls nitrate fate along advective flow paths, with local carbon availability and residence time jointly determining pathway dominance [44].

Similarly, anammox and denitrifiers compete for nitrite, influencing total N_2 production efficiency [28]. The partitioning between these pathways is further modulated by dissolved organic carbon (DOC) concentration: when DOC: NO_3^- ratios exceed critical thresholds, heterotrophic denitrification outcompetes anammox for available nitrite, whereas under carbon-limited conditions anammox maintains a proportionally larger contribution to nitrogen loss [33].

Hydrological exchange acts as a key regulatory driver of these interactions by controlling residence time, oxygen intrusion, and substrate delivery. Column experiments simulating hyporheic exchange demonstrate that denitrification, DNRA, and nitrification rates change rapidly in response to shifts in water chemistry and fluid residence time, with microbial functional potential toward denitrification correlating with steep NO_3^- concentration gradients along flow paths [1]. High-frequency flow reversals enhance microbial diversity and promote functional redundancy, while longer residence times favor complete nitrogen transformation sequences [47,58]. Grain size heterogeneity further modulates these dynamics by controlling pore-scale flow velocities and oxygen penetration depths, with finer sediments supporting higher DNRA and anammox activity due to prolonged anaerobic conditions [10,11].

Emerging functional groups such as comammox and N-DAMO act as network modifiers, altering substrate availability and reshaping interaction hierarchies within nitrogen cycling networks [18,43]. These organisms reduce the modular separation of classical nitrogen pathways, increasing connectivity within the microbial nitrogen cycle. In particular, comammox *Nitrospira* eliminates the spatial dependency between ammonia oxidation and nitrite oxidation, potentially tightening the coupling between nitrification and downstream reductive pathways in oxygen-limited microsites [12].

Collectively, HRZ microbial communities exhibit high functional redundancy and metabolic plasticity, allowing rapid reorganization of nitrogen transformation pathways in response to environmental perturbations [46,50]. This plasticity is evidenced by the observation that microbial community functional potential shifts within days of hydrological regime changes, with zonation of denitrification functional genes established along 30 cm advective flow paths after only 6 days of groundwater elution [1].

4. Molecular approaches for investigating nitrogen cycling in HRZs

Understanding nitrogen cycling in HRZs requires integrative methodologies capable of linking microbial identity, functional potential, and realized process rates across spatially heterogeneous environments. Traditional culture-based approaches are insufficient due to the strong decoupling between microbial presence and activity in dynamic redox systems [7,60]. The methodological evolution in this field reflects a progressive shift from descriptive taxonomic inventories toward mechanistic, process-resolved frameworks that integrate molecular, isotopic, and hydrodynamic information [13].

4.1. Functional gene analysis: role and limitations

PCR-based functional gene analysis (e.g., nifH, amoA, nirS, nirK, nrfA, hzsA) remains a foundational tool for identifying nitrogen-transforming microbial communities [51]. In riparian zone sediments, the abundances of functional genes (norB, nirS, nrfA) (Figure 3), and anammox bacterial 16S rRNA gene show distribution patterns that correlate with corresponding nitrogen transformation rates, establishing gene abundance as a first-order indicator of pathway potential [3]. However, gene abundance reflects potential rather than actual process rates, particularly in HRZs where microbial activity is highly responsive to hydrological and geochemical fluctuations [53]. Consequently, functional gene data are increasingly interpreted as indicators of metabolic capacity rather than direct proxies of nitrogen flux.

Several methodological and conceptual limitations constrain the interpretive power of functional gene analysis: Gene abundance does not reliably correlate with enzymatic activity or nitrogen transformation rates, particularly in environments characterized by rapid redox oscillations [53,55]. In hyporheic zone column experiments, shifts in microbial functional potential were resistant to short-term groundwater–surface water exchange on daily timescales, while longer-term shifts occurred over days to weeks [1], indicating temporal lags between genetic potential and realized function. Spatial heterogeneity in HRZ sediments complicates extrapolation of point-based gene measurements to ecosystem-scale fluxes. Nitrogen cycling processes occur over microscale gradients that are not adequately captured by bulk sediment sampling [53]. Biofilm-induced bioclogging can produce sharp interfaces in redox conditions and microbial community structure within centimeter-scale distances [14], generating spatial discontinuities that point measurements cannot resolve. Methodological biases in primer specificity and amplification efficiency may underestimate microbial diversity, particularly for novel or poorly characterized nitrogen-cycling taxa [51]. Additionally, DNA-based approaches cannot distinguish between active and inactive microbial populations, potentially overestimating functional capacity in sediment systems where relic DNA persists [54].

RNA-based approaches, including RT-qPCR and metatranscriptomics, partially address these limitations by targeting gene expression rather than gene presence. However, these methods remain technically challenging due to RNA instability and extraction constraints in complex environmental matrices [49].

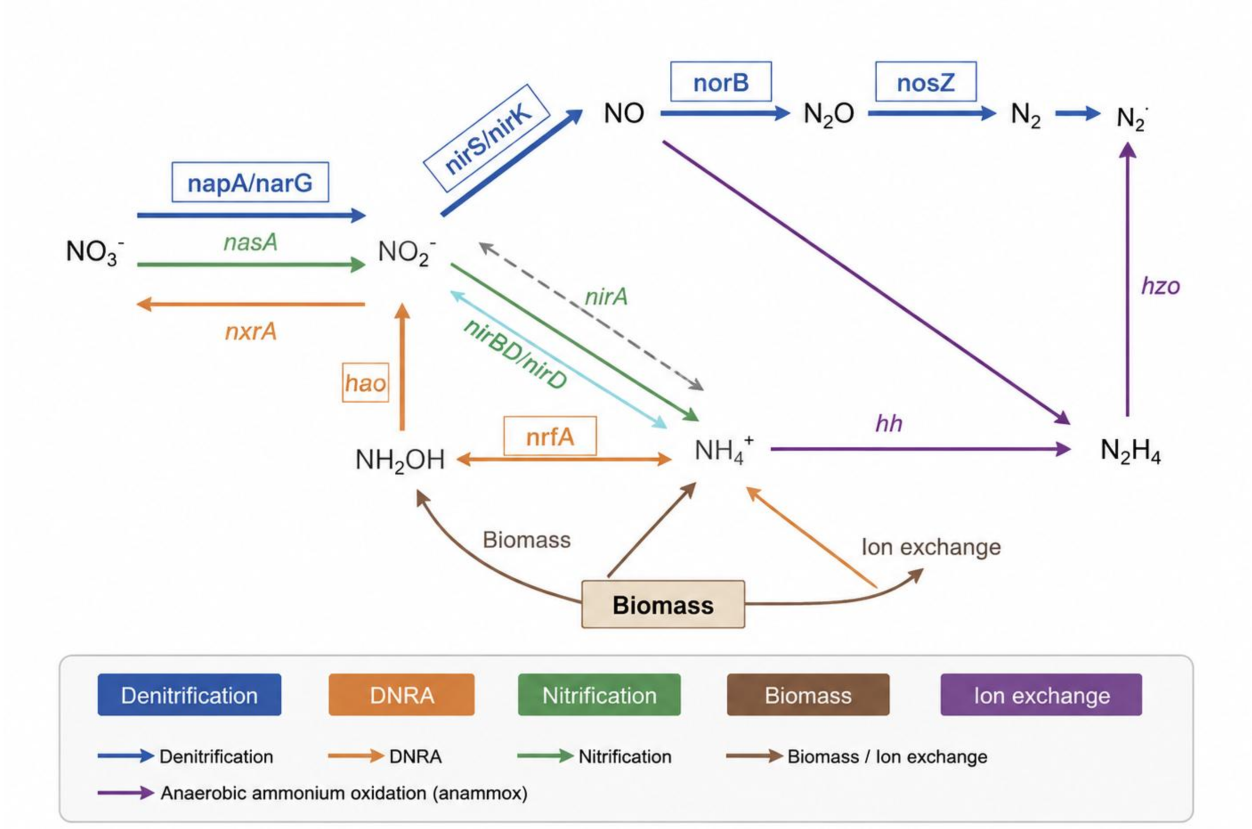

Figure 3. Functional gene markers associated with major nitrogen transformation pathways in HRZ microbial communities: This schematic illustrates key microbial nitrogen transformation pathways, including denitrification, dissimilatory nitrate reduction to ammonium (DNRA), nitrification, biomass incorporation, and ion exchange. Color-coded arrows denote the specific biochemical routes and enzymes involved, such as nitrate reductases (napA/narG, nasA), nitrite reductases (nirS/nirK, nirA, nirBD/nirD), nitric oxide reductase (norB), nitrous oxide reductase (nosZ), hydroxylamine oxidoreductase (hao), nitrite reductase (nrfA), hydrazine synthase (hh), and hydrazine oxidoreductase (hzo). This professional, review-style figure emphasizes mechanistic clarity of nitrogen cycling at the microbial level.

4.2. Integrated multi-omics and isotopic approaches

High-throughput sequencing approaches, including metagenomics and metatranscriptomics, provide deeper insights into the functional organization of nitrogen cycling communities. These methods reveal that HRZ nitrogen transformations are driven by metabolically flexible microbial assemblages capable of shifting between aerobic and anaerobic pathways depending on redox conditions and substrate availability [52]. Metatranscriptomic data further allow identification of actively expressed nitrogen cycling pathways, enabling temporal resolution of process activation that static DNA-based surveys cannot achieve.

Stable isotope techniques represent a major advancement in quantifying nitrogen transformation rates. ^{15}N-based tracer experiments, stable isotope probing (SIP), and compound-specific isotope analysis (CSIA) enable direct measurement of process-specific fluxes such as denitrification, DNRA, and anammox in situ [56, 57]. Application of ^{15}N tracing in riparian sediments has enabled simultaneous quantification of denitrification, DNRA, and anammox rates within the same sediment profile, revealing that when NO_3^- content falls below 0.06 mg/kg, DNRA occurs preferentially over denitrification [3]. When combined with hydrological residence time modeling and porewater chemistry, these methods allow partitioning of gross nitrogen transformation into pathway-specific contributions, reducing uncertainty in ecosystem-scale nitrogen budgets [58].

A major conceptual advancement in HRZ research is the integration of molecular datasets with hydrological and reactive transport models. Mathematical frameworks coupling hydrodynamics, solute transport, and microbial growth processes describe nitrogen transport and transformation behavior in the hyporheic zone, with coupling relationships among these models enabling prediction of spatially distributed nitrogen fate [13]. This integration enables scaling of microscale microbial activity to reach-scale nitrogen fluxes, addressing a key limitation of purely genetic approaches [60]. However, robust predictive capacity still depends on improved parameterization linking gene expression to process rates under dynamic environmental conditions. Current challenges include uncertainty arising from experimental data acquisition, process coupling relationship establishment, and model parameter identification [13].

5. Challenges and knowledge gaps in understanding nitrogen cycling in HRZs

Despite substantial advances in understanding nitrogen transformation processes in hyporheic–riparian zones, major uncertainties remain regarding the mechanisms controlling nitrogen retention, removal, and export across spatial and temporal scales.

5.1. Environmental heterogeneity and scaling challenges

A persistent challenge is the extreme heterogeneity of HRZ environments, where hydrological exchange, sediment structure, redox gradients, and organic matter availability vary over very short distances, generating highly dynamic microbial niches and transformation hotspots [4,12,59]. The concept of "hot spots and hot moments" captures this spatiotemporal variability, wherein nitrogen removal is concentrated at specific locations and time periods rather than distributed uniformly across the HRZ [9]. This heterogeneity complicates extrapolation of point-scale observations to ecosystem- or watershed-scale nitrogen budgets.

Biofilm dynamics add further complexity: biofilm-induced bioclogging produces sharp interfaces in hyporheic flow, creating abrupt transitions in redox conditions and microbial community structure that generate discontinuous reaction zones within otherwise continuous sediment matrices [14]. Ambient groundwater flow further diminishes nitrate processing capacity by reducing hyporheic exchange volumes and shortening effective reaction path lengths [15]. These physical controls impose constraints on nitrogen processing that are independent of microbial community composition.

5.2. Process overlap and pathway discrimination

Current understanding of HRZ nitrogen cycling is constrained by the difficulty of resolving simultaneous and overlapping microbial processes. Denitrification, DNRA, anammox, and emerging pathways such as sulfammox and feammox often co-occur within adjacent sediment microzones and compete for shared intermediates including nitrate and nitrite [2,26,34]. In riparian zone sediments, non-rhizosphere zones reinforce contributions of feammox and anammox to nitrogen loss relative to rhizosphere zones, indicating that root-mediated oxygen release and carbon exudation differentially modulate pathway expression across sub-centimeter gradients [16]. Conventional analytical methods frequently fail to distinguish these pathways quantitatively, leading to uncertainty in estimates of net nitrogen retention versus permanent nitrogen removal [62].

The competition between heterotrophic denitrification and DNRA exemplifies this discrimination challenge. Modeling studies demonstrate that competitive outcomes are spatially explicit and sensitive to local moisture, redox conditions, and organic nitrogen dynamics in partially saturated soil–water systems [8], yet field validation of these predictions remains limited. Under nitrate-limited conditions, DNRA holds a competitive advantage over denitrification [4], but predicting when and where this advantage manifests in heterogeneous HRZ environments requires coupling of microbial kinetics with spatially resolved transport models [5].

5.3. Linking functional potential to realized ecosystem function

Although molecular approaches have significantly improved characterization of nitrogen-transforming microbial communities, the relationship between gene abundance, gene expression, and actual process rates remains poorly constrained [51,53]. In HRZs, rapid fluctuations in oxygen availability, residence time, and substrate delivery can decouple microbial presence from metabolic activity, limiting the predictive power of gene-based analyses alone [47,55]. A regulation-structured dynamic metabolic model for denitrification in hyporheic zone sediments revealed significant time lags (up to several days) in enzymatic response to changes in substrate concentration, demonstrating that even when genetic potential is present, kinetic delays create temporal decoupling between environmental forcing and process rates [17].

This challenge is particularly important when attempting to integrate microbial datasets into hydrological and reactive transport models. Current coupled models describe nitrogen transport and transformation behavior but face persistent uncertainty arising from process coupling relationships and parameter identification [13]. Without improved mechanistic understanding of gene expression–activity relationships, predictive models will remain underconstrained.

5.4. Temporal dynamics and hydrological forcing

Seasonal water-level fluctuations exert depth-dependent control on microbial nitrogen transformation and greenhouse gas fluxes in riparian zones, creating temporally variable process regimes that are poorly captured by snapshot sampling [18]. Single flood events can restructure hyporheic nitrogen processing by altering bedform-flow interactions and modifying nitrate removal efficiency over event timescales [19]. The production and emission of N_2O—a potent greenhouse gas from hyporheic zones is further regulated by ripple migration dynamics that create transient reaction zones with incomplete denitrification [20].

River stage fluctuations induced by tides, dam releases, or storms enhance nitrogen cycling in riparian zones by periodically saturating and draining sediment pores, creating oscillating redox conditions that stimulate coupled nitrification–denitrification sequences [21]. These temporal dynamics mean that time-averaged measurements substantially underestimate peak nitrogen processing rates and may mischaracterize the dominant transformation pathways operating during hydrological events.

5.5. Emerging pathways and multi-element coupling

Knowledge gaps are especially pronounced for emerging and alternative nitrogen transformation pathways. Processes such as comammox, N-DAMO, sulfammox, and feammox are increasingly recognized in sedimentary environments, yet their ecological significance, environmental thresholds, and quantitative contribution to HRZ nitrogen cycling remain insufficiently understood [30,32,35,61]. In particular, the coupling of nitrogen cycling with sulfur, iron, and methane dynamics introduces additional layers of complexity that are rarely incorporated into conceptual or numerical models. Fe(II) promotes NO_3^- attenuation through nitrate-dependent ferrous oxidation under microbial mediation, while DOC enhances attenuation through DNRA [3], demonstrating that multi-element interactions are not merely additive but create synergistic or antagonistic effects on nitrogen fate.

Sediment grain size introduces an additional physical control on these coupled processes by regulating nutrient concentrations and microbial abundance within the reaction zone [11,10]. Finer-grained sediments promote anaerobic conditions conducive to DNRA and anammox, while coarser sediments maintain higher dissolved oxygen concentrations favoring nitrification and aerobic respiration.

5.6. Anthropogenic disturbance and climate change

Climate change and anthropogenic disturbance introduce further uncertainty into HRZ nitrogen dynamics. Altered precipitation regimes, increased groundwater fluctuations, nutrient enrichment, channel modification, and land-use intensification can substantially modify redox structure, hydrological residence time, and microbial community composition [36,37,50]. Pesticide accumulation in riparian zone soils, an overlooked anthropogenic stressor—impacts nitrogen-cycling-related microbial communities, with acetochlor exposure altering both nitrification and denitrification functional groups over 60-day timescales [22]. In-stream structures designed for ecological restoration significantly change nitrogen transport and biochemical processes in the hyporheic zone, representing a direct anthropogenic modification of nitrogen cycling capacity [23].

These disturbances may shift the balance between nitrogen retention and nitrogen loss pathways, potentially reducing the buffering capacity of HRZs against downstream eutrophication. Long-term and geographically diverse datasets are also lacking: most HRZ nitrogen cycling studies are concentrated in temperate systems, while tropical, arid, and heavily modified river corridors remain comparatively understudied [22].

6. Conclusion and future perspective

Hyporheic–riparian zones function as spatially structured, temporally dynamic biogeochemical reactors whose nitrogen processing capacity is an emergent property of coupled physical and microbial interactions across nested scales. Nitrogen cycling within these zones arises from interconnected microbial pathways including nitrification, comammox, denitrification, DNRA, anammox, N-DAMO, sulfammox, and feammox whose competitive and syntrophic interactions, modulated by hydrological residence time, redox gradients, sediment heterogeneity, and organic carbon availability, determine whether HRZs act as sinks, transformers, or sources of nitrogen.

Future research should prioritise integrating multi-omics, high-resolution isotope tracing, and reactive transport modelling to mechanistically resolve nitrogen transformations under fluctuating environmental conditions. Event-resolved monitoring, predictive modelling, and consideration of anthropogenic and climate-driven perturbations are essential for understanding the threshold dynamics, microbial bistability, and quantitative contribution of emerging pathways. Such integrative approaches will inform sustainable watershed management strategies that leverage the intrinsic nitrogen-processing potential of HRZs while anticipating the impacts of global environmental change

Author’s Contributions

S.B. A.Y: Conceptualization, Methodology, Literature Review, Data Curation, Visualization, and Project Administration. **A.Y.:** Supervision, Original Draft Preparation, Critical Review and Editing of Manuscript, Methodological Guidance, Validation, and Resources.

A.A.: Critical Review and Editing of Manuscript. **F.O.:** Review of the final draft of the manuscript. **A.M.A.:** Critical review and editing. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Conflicts of Interest

The authors declare that there is no commercial or financial conflict of interest that could be perceived as influencing the content of this article.

Data Availability

All data and materials supporting the findings of this study are included within the article. No new datasets were generated or analyzed during the current study. Additional information and resources are available from the corresponding author upon reasonable request.

Funding

This research received no external funding.

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