

Review

The Potential of Biochar in Wastewater Denitrification: Mechanisms, Redox-Mediated Electron Transfer, and Advanced Modifications

Yangyang Wang ^{1,2,†}, Shengnan Lv ^{1,2,†}, Haochun Zang ¹, Shuhu Xiao ¹, Liangjie Wang ³, Haiya Zhang ¹ and Bingfei Yan ^{1,4,*}

¹ State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China; yangwang4511@163.com (Y.W.); 18838020304@163.com (S.L.); zanghch@126.com (H.Z.); xiaoshuhu@126.com (S.X.); flying850612@126.com (H.Z.)

² College of Environmental Engineering, Henan University of Technology, Zhengzhou 450001, China

³ The Key Laboratory of Water and Sediment Sciences (Ministry of Education), College of Environmental Sciences and Engineering, Peking University, Beijing 100871, China; wljcumt@126.com

⁴ College of Water Sciences, Beijing Normal University, Beijing 100875, China

* Correspondence: ybf201506@163.com

† These authors contributed equally to this work.

Abstract

Biochar has attracted increasing attention for aquatic pollution control, particularly due to its capacity to accelerate the rate-limiting steps of denitrification in wastewater treatment. While early research primarily focused on the adsorption capacity of biochar, recent studies have increasingly investigated its role as a redox-active mediator that facilitates electron transfer. This review critically synthesizes the multifaceted mechanisms of biochar-enhanced denitrification, establishing a link between synthesis parameters (feedstock, pyrolysis kinetics) and physico-chemical functionalities (pore architecture, redox-active functional groups). Specifically, we elucidate how precise regulation of pyrolysis temperature dictates the dominant electron transfer pathway: low-temperature biochar (<500 °C) facilitates electron shuttling via oxygen-containing functional groups (e.g., quinone moieties), whereas high-temperature biochar (>700 °C) promotes direct interspecies electron transfer (DIET) through graphitic conduction. We systematically decouple biochar-mediated electron transfer into three pathways: functional group-driven shuttling, solid-state conductive matrix transfer via conjugated π -electrons, and material-assisted DIET. Crucially, we emphasize that validating true DIET requires direct biological evidence of electroactive machinery. Furthermore, the review details how biochar modulates the biological microenvironment, upregulating key denitrification genes (*narG*, *nirS/K*, *nosZ*) and enriching functional microbial consortia. By integrating advances in surface modification—such as heteroatom doping and metal loading—we propose strategies to engineer biochar for optimized nitrate-to-nitrogen conversion. Future perspectives underscore the need for balancing electron-donating capacity with structural stability, developing low-energy functionalization techniques, and conducting life-cycle assessments to facilitate the scale-up of sustainable, high-efficiency nitrogen removal systems.

Keywords: biochar; electronic transmission; nitrogen removal; denitrification



Academic Editors: William Frederick Ritter and Catherine N. Mulligan

Received: 19 May 2026

Revised: 16 June 2026

Accepted: 13 July 2026

Published: 22 July 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\) license](https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The escalating discharge of nitrogenous contaminants into aquatic ecosystems—driven by rapid urbanization and agricultural intensification—poses a severe threat to global wa-

ter security, manifesting in eutrophication and the destabilization of aquatic biodiversity. Biological denitrification, the conventional terminal step in nitrogen removal, is often constrained by insufficient carbon sources, slow electron transfer kinetics, and the accumulation of toxic intermediates like nitrite and nitrous oxide (N₂O). Consequently, there is an urgent imperative to develop cost-effective, sustainable materials that can catalyze this biochemical process.

Biochar, a carbon-rich solid produced via biomass pyrolysis, has evolved from a traditional soil amendment (“Terra Preta”) to a sophisticated functional material for environmental remediation [1–3]. While early literature was dominated by studies on its physicochemical adsorption properties due to large specific surface area and porosity, mere physical adsorption is often limited by saturation risks [4,5]. Therefore, recent research frontiers have pivoted towards exploiting biochar’s unique redox-active nature. In this capacity, biochar demonstrates an “electron-buffering” ability, acting as both an electron donor and a shuttle to accelerate the rate-limiting steps of nitrate reduction. This paradigm shift is substantiated by recent publication trends (2010–2025), which reveal a sharp upward trajectory in biochar-enhanced denitrification research, signaling that this domain is rapidly gaining academic prominence (Figure 1).

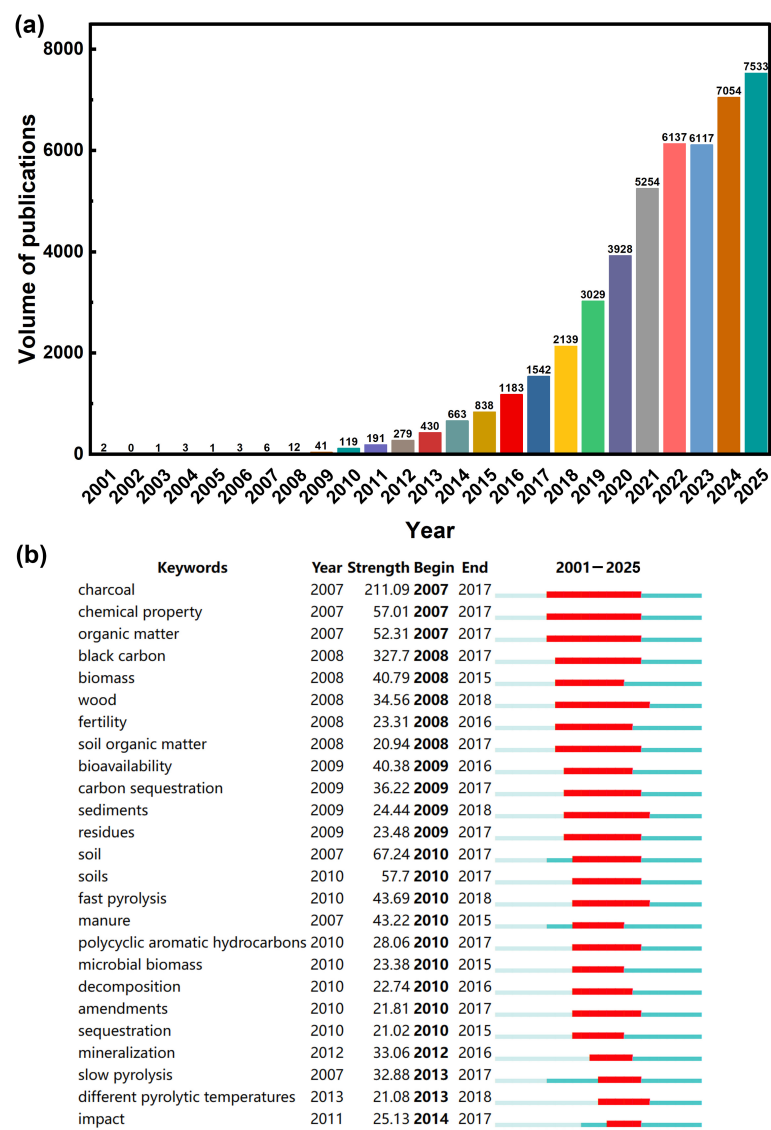


Figure 1. Cont.

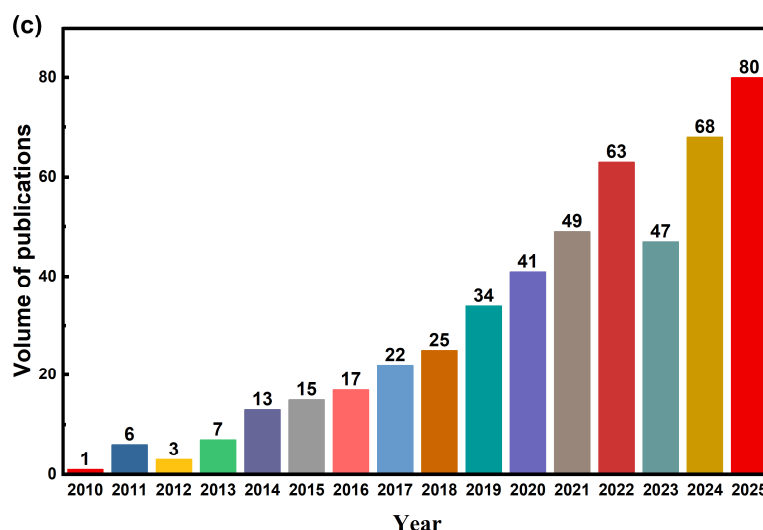


Figure 1. Bibliometric analysis of global research trends in biochar and denitrification. (a) Annual publication volume of biochar research from 2001 to 2025. (b) Top 25 keywords with the strongest citation bursts (2001–2025). (c) Annual publication volume specifically focusing on biochar-enhanced denitrification (2010–2025).

Despite this surging interest, a comprehensive understanding of the structure–function relationships governing biochar-mediated electron transfer remains fragmented, leading to a persistent ambiguity in contemporary literature regarding the precise boundaries among redox mediation, conductive electron transfer, and true DIET. Currently, these terms are frequently used interchangeably without rigorous biophysical distinction, obscuring two critical knowledge gaps: (1) the mechanistic dichotomy between biochar’s role as a non-conductive redox mediator (driven by oxygen-containing functional groups) versus a conductive solid-state electron channel (enabled by graphitic structures); and (2) the complex interplay between biochar surface chemistry and the genetic regulation (e.g., *narG* and *nosZ* expression) of denitrifying consortia. To bridge these gaps and provide a clearer mechanistic framework for the scientific community, this review critically differentiates between functional group-based electron shuttling, carbonaceous matrix-driven conduction, and true biological DIET, highlighting the specific electrochemical and transcriptomic evidence required to validate each pathway during wastewater denitrification.

This review aims to bridge these gaps by critically synthesizing the latest advances in biochar-enhanced denitrification. We analyze how pyrolysis kinetics tailor redox properties, dissect the distinct mechanisms of electron transfer, and evaluate advanced modification strategies. These mechanisms provide a theoretical foundation for designing efficient biochar-based systems for advanced wastewater treatment.

2. Biochar Preparation

Feedstock selection, pyrolysis technologies, and processing conditions constitute three pivotal determinants in biochar production, directly governing its physicochemical properties. These property modifications critically regulate biochar’s adsorption capacity, catalytic activity, and reaction kinetics during denitrification, thereby influencing nitrogen oxide removal efficiency and operational stability. Ultimately, the synergistic effects of these fabrication parameters dictate biochar’s quality metrics—including reactivity, durability, and environmental compatibility—which fundamentally constrain its practical applications across wastewater treatment, soil remediation, and energy storage domains.

2.1. Biochar Feedstock

The physicochemical properties of biochar, which fundamentally dictate its regulatory effects on microbial denitrification and greenhouse gas (GHG) mitigation, are highly dependent on the selection of biochar feedstocks. Different raw materials imbue biochar with distinct pore structures, functional group compositions, and labile carbon fractions, thereby driving divergent pathways in nitrogen cycling.

Generally, agricultural residues, such as crop straws and cornstalks, are rich in lignocellulose. Crop-straw-derived biochars have been demonstrated to substantially stimulate complete soil denitrification under field conditions, effectively mitigating gaseous nitrogen loss by accelerating the reduction of N_2O to N_2 [6]. Moreover, pristine cornstalk biochar possesses active surface-bound phenolic and quinone functional groups; these redox-active centers can enhance cell growth and boost cellulose degradation rates, thereby increasing the bioavailability of ambient carbon sources for denitrifying consortia [7].

In contrast, manure-based biochars (e.g., dairy or poultry manure) typically retain a higher proportion of easily mineralizable carbon elements. When pyrolyzed at relatively low temperatures, manure biochars exhibit high aliphaticity and abundant dissolved organic carbon (DOC), which provide a superior electron-donating capacity (EDC) to modulate soil N_2O production fluxes and upregulate microbial nitrogen-cycling genes [8]. Furthermore, when paired with organic matrices like food waste digestate, biochar can successfully trigger completely coupled denitrification and anaerobic methane oxidation (AOM) processes in specific environments like paddy soils [9].

Lastly, sewage sludge-derived biochars have gained increasing attention in engineering wastewater treatment systems due to their unique structural advantages. The highly porous skeleton of sludge biochar serves as an ideal carrier for biofilm attachment, promoting extracellular polymeric substance (EPS) secretion and ensuring stable, long-term carbon slow release for heterotrophic denitrification in sequencing batch reactors (SBRs) [10]. Notably, the surface functional groups of modified sludge biochars can function as effective “geoconductors” or solid electron shuttles, directly accelerating extracellular electron transfer to microbial cells and markedly boosting nitrate removal rates [11].

To provide a comprehensive benchmarking overview, the diverse application scenarios, performance matrices, and underlying mechanisms of biochar-mediated denitrification based on these representative feedstock categories are systematically summarized in Table 1.

Table 1. Comparison of application scenarios, performance, and mechanisms of biochar-mediated denitrification and GHG mitigation based on representative literature.

Biochar Type and Feedstock	Application Scenario	Key Denitrification/Mitigation Performance	Main Underlying Mechanisms	References
Food waste digestate paired with biochar	Agricultural and paddy soils	Significant reduction in cumulative N_2O and CH_4 emissions from rice paddy soil.	Boosted completely coupled denitrification and AOM.	[9]
Municipal sewage sludge-derived biochar/PHBV composite	Acidic/alkaline and tea soils	Achieved stable long-term carbon release and enhanced heterotrophic nitrogen removal.	Provided ideal porous attachment carrier for biofilm; promoted EPS secretion.	[10]
Cornstalk-derived biochar applied at $5 \text{ g} \cdot \text{L}^{-1}$	DNRA and cocontamination	Nitrate removal efficiency reached 96.48% within 48 h with negligible NO_2^- accumulation.	Active phenolic and quinone functional groups accelerated microbial cell growth and cellulose degradation.	[7]
Crop-straw-derived biochar	Constructed wetlands and bioretention	Substantial decreases in both annual N_2O and NO soil emissions under field conditions.	Mitigated gaseous nitrogen loss by stimulating the reduction of N_2O to N_2 through enhanced denitrification.	[6]

Table 1. Cont.

Biochar Type and Feedstock	Application Scenario	Key Denitrification/Mitigation Performance	Main Underlying Mechanisms	References
Manure-derived biochar (dairy/poultry manure)	Anammox/SBR/AD reactors	Modulated soil N ₂ O production fluxes via labile carbon fraction inputs.	Low-temperature biochar with high aliphaticity and DOC provided strong EDC.	[8]
Melamine-modified sewage sludge biochar	Engineering denitrification systems	Greatly accelerated nitrate removal rates through enhanced electron transfer.	Biochar surface functional groups acted as a “geoconductor” to shuttle electrons directly to microbes.	[11]

Note: PHBV, poly(3-hydroxybutyrate-co-3-hydroxyvalerate).

2.2. Pyrolysis Technology

Although biochar synthesis utilizes a diverse array of feedstocks—ranging from agricultural residues to sludge—the final physicochemical architecture is predominantly dictated by the pyrolysis regime rather than the precursor material itself. While the feedstock provides the elemental foundation (lignin, cellulose, and ash), it is the thermal conversion parameters that fundamentally engineer the biochar’s pore structure, surface functionalization, and redox capabilities. Consequently, the selection of pyrolysis technology—categorized by processing mechanisms (microwave, hydrothermal) or heating rates (slow, intermediate, fast)—serves as the primary lever for tailoring biochar’s environmental behavior and denitrification efficacy (Table 2).

Table 2. Description of different thermal conversion processes.

Pyrolysis Technology	Pyrolysis Temperature	Pyrolysis Time	Heating Rate	Biochar Yield	Reference
Slow pyrolysis	400–600 °C	1 h	5–7 °C/min	35%	[2,4,12]
Intermediate pyrolysis	400–600 °C	10–20 s	200 °C/min	20%	[13,14]
Rapid pyrolysis	500–600 °C	1 s	200 °C/min	10%	[12,15]
Microwave pyrolysis	400–1000 °C	0.3–9 h	–	35–50%	[5]
Hydrothermal carbonization	180–250 °C	1–12 h	–	30–60%	[12,16]

Pyrolysis methodologies are classified into slow, intermediate, and fast pyrolysis based on heating rates and residence times, each yielding distinct product distributions. Slow pyrolysis operates within relatively low temperature ranges (400–600 °C) with extended reactor residence times (hours), facilitating thorough biomass decomposition and higher biochar yields. Intermediate pyrolysis, as a balanced approach, employs comparable temperatures (400–600 °C) but shorter residence times (minutes to tens of minutes), achieving equilibrium between biochar and liquid product generation. In contrast, fast pyrolysis utilizes rapid heating rates and brief residence times (seconds) at elevated temperatures (>500 °C), primarily yielding bio-oil and volatile gases [17], with minimal biochar production. These pyrolysis strategies fundamentally dictate biochar’s critical attributes, including pore structure development (micropores, mesopores, and macropores), specific surface area magnitude, and surface functional group diversity/abundance (e.g., hydroxyl and carboxyl groups). Consequently, these properties govern biochar’s denitrification performance: pore architecture provides microbial habitats, expanded surface area increases reactive sites, and functional groups mediate electron transfer and catalytic reactions. Optimizing pyrolysis parameters (temperature gradients, residence times, and heating rates) enhances biochar’s hydrophilicity and surface area, which synergistically improve microbial attachment, electron transport efficiency, and metabolic activity of denitrifying bacteria (e.g., *Pseudomonas* spp.). This optimization strategy significantly boosts nitrate removal rates [13,18,19] and

operational stability, establishing biochar as a pivotal material in wastewater treatment and environmental remediation applications.

Microwave pyrolysis is a technology that utilizes microwave energy to efficiently heat biomass feedstocks through “dielectric heating” or “molecular friction” mechanisms. During the heating process, microwaves rapidly penetrate the biomass matrix, enabling uniform heat distribution at exceptional rates to achieve homogeneous bulk heating [5]. Compared to conventional pyrolysis, this method produces biochar with more uniform morphology, compact structure, and significantly lower energy consumption. However, under identical temperature conditions, microwave pyrolysis generally yields less biochar than slow pyrolysis, which limits its potential in high-yield applications requiring substantial carbon retention.

Hydrothermal carbonization (HTC) is an exothermic reaction process conducted in a high-pressure aqueous environment, where feedstocks are converted into biochar-dominated products alongside high-value derivatives such as liquid fuels and gaseous byproducts [20]. HTC exhibits superior biomass-to-biochar conversion rates and enhanced carbon sequestration efficiency compared to conventional methods, while operating at relatively low temperatures (180–250 °C), thereby reducing energy demands and mitigating equipment corrosion risks. Furthermore, this approach enables direct processing of wet biomass feedstocks without requiring pre-drying pre-treatment steps.

Microwave pyrolysis and hydrothermal carbonization exhibit distinct operational emphases in biochar production: the former prioritizes heating efficiency and product uniformity, while the latter emphasizes conversion efficiency and mild processing conditions. The optimization of biochar’s physicochemical properties—particularly porous architecture and surface functional group configuration—is critically governed by method selection and parameter control, including temperature, pressure, feedstock characteristics, and reaction duration. This precision engineering proves vital for developing high-performance denitrification biochar, as its environmental remediation capability fundamentally relies on maximized specific surface area and enhanced adsorption capacity to effectively immobilize and convert nitrate contaminants through synergistic physicochemical mechanisms.

2.3. Pyrolysis Temperature

The principal lignocellulosic components of biomass feedstocks—cellulose, lignin, and hemicellulose—exhibit distinct thermal decomposition behaviors due to their heterogeneous chemical architectures, establishing pyrolysis temperature as the critical determinant of biochar’s physicochemical properties [21]. Elevated pyrolysis temperatures enhance carbonization completeness, aromatic matrix formation, and structural stabilization, albeit at the expense of reduced biochar yield and increased feedstock carbon loss [22]. Comparative studies using oxygen-limited pyrolysis of rice straw demonstrate that high-temperature biochars develop superior pore architectures and enlarged specific surface areas, characteristics typically advantageous for adsorption processes [23]. Paradoxically, such biochars exhibit diminished electron transfer efficiency in denitrification systems due to thermal degradation of oxygenated functional groups (e.g., carboxyl, hydroxyl, and phenolic–OH), which are essential as redox mediators. The optimal electron-donating capacity in low- and medium-temperature biochars is attributed to preserved surface functionalities that facilitate interspecies electron transfer between denitrifying microbes and biochar interfaces. In contrast, high-temperature biochars suffer from reduced electron mediation capacity despite enhanced graphitization, as oxygen-containing functional groups critical for electron shuttling are thermally decomposed [24]. Furthermore, the composition and abundance of surface functional groups directly regulate the activity of denitrification-associated en-

zymes (e.g., nitrate reductase, nitrite reductase), thereby influencing overall denitrification efficiency [25].

Thermal processing variability induces complex chemical transformations (e.g., cracking, polymerization) with divergent reaction pathways, resulting in substantial heterogeneity in biochar's physicochemical characteristics, including specific surface area, pore size distribution, and surface functional group composition. This variability necessitates meticulous optimization of production protocols through controlled temperature ranges, precise residence time modulation, and tailored feedstock formulations to engineer structurally stable biochar with enhanced adsorption performance.

3. Biochar Modification Methods

Biochar is structurally categorized into four principal domains (Figure 2): aromatic carbon matrices, hierarchical pore architectures, surface functional group configurations, and mineral precipitate inclusions. Current modification strategies predominantly target pore structure optimization and functional group engineering to enhance specific physicochemical functionalities.

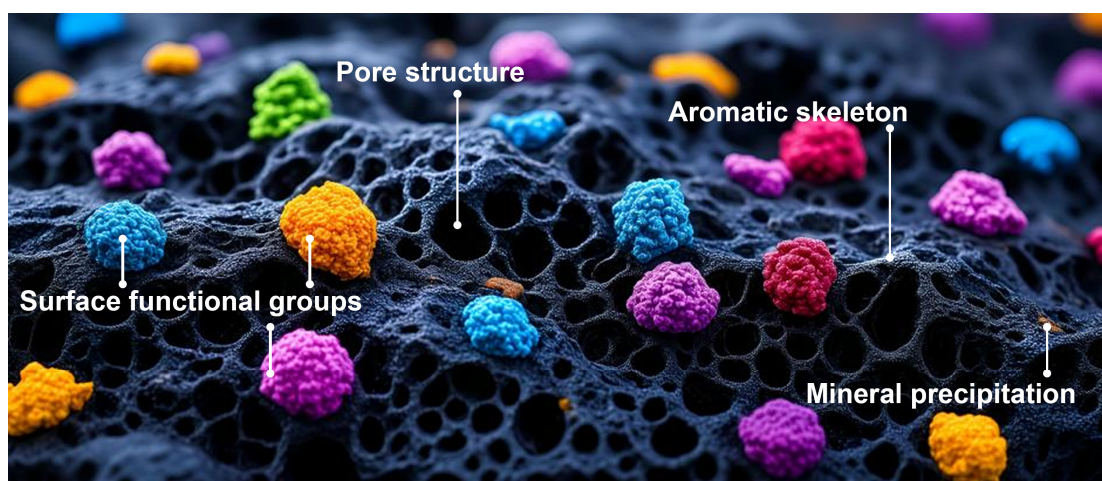


Figure 2. Structure composition of biochar.

3.1. Pore Regulation

Controllable porous architecture and elevated specific surface area are critical for optimizing adsorption capacity and catalytic activity in carbon-based materials during pollutant removal processes, particularly for denitrification applications. The enhanced surface area facilitates increased reactive site availability for contaminant immobilization and transformation, while optimized pore configuration improves molecular diffusion kinetics and reaction dynamics, collectively determining material performance in denitrification systems [26]. Conventional biochar synthesized through direct pyrolysis exhibits constrained adsorption sites and suboptimal porosity, resulting in insufficient contaminant uptake efficiency when treating high-concentration pollutants and limited applicability for advanced denitrification requirements [27].

Conventional strategies for pore structure modulation primarily involve in situ regulation during pyrolysis and post-carbonization modification. In situ regulation refers to the direct control of biochar's pore architecture and specific surface area through parameter optimization during biomass carbonization, including pyrolysis temperature (200–700 °C), heating rate, and atmospheric conditions (e.g., inert nitrogen or oxygen levels) [28]. Corresponding investigations [23] demonstrated that high-temperature pyrolysis (>500 °C) promotes the formation of microporous and mesoporous networks, significantly enhancing

specific surface area from $1.84 \text{ m}^2/\text{g}$ to over $881 \text{ m}^2/\text{g}$. This structural evolution increases reactive site density and nitrate adsorption capacity, achieving 549–793% efficiency improvement through enhanced interfacial interactions. In contrast, low-temperature-derived biochar exhibits underdeveloped porosity and limited adsorption performance.

Elevated pyrolysis temperatures induce thermal decomposition of surface functional groups (e.g., carboxyl and hydroxyl), compromising the material's electron transfer capacity and nitrogen removal activity, which explains the paradoxical decline in denitrification efficiency despite enhanced adsorption performance in high-temperature biochars [29]. This phenomenon necessitates secondary modification strategies for carbon materials to achieve balanced adsorption–stabilization characteristics, particularly crucial for optimizing denitrification processes through preserved redox-active surface functionalities.

Secondary activation refers to post-synthesis modification of biochar through physicochemical methods at elevated temperatures, designed to enhance adsorption capacity via controlled pore structure engineering. Compared to primary pyrolysis, this process enables precise development of porous architectures with increased micropore (<2 nm) and mesopore (2–50 nm) densities, thereby optimizing functional performance. Two principal activation pathways exist: physical activation employs oxidizing agents (CO_2 , H_2O , air, or their mixtures) at 800–900 °C to selectively remove the carbon matrix through gasification, generating microporous structures advantageous for small-molecule contaminant adsorption [30,31]. However, this method requires prolonged thermal exposure (typically several hours) with high energy consumption and limited scalability [32]. Chemical activation offers superior efficiency through impregnation with agents like HCl or NaOH, followed by thermal treatment to simultaneously introduce functional groups and expand porosity [33]. NaOH-modified biochar is demonstrated to achieve rapid ammonium adsorption equilibrium (<10 h) with exceptional capacity (>200 mg/g) across broad pH/temperature ranges, attributed to enhanced surface electronegativity and ion-exchange mechanisms [34]. Similarly, HCl-activated biochar derived from mixed feedstocks (650 °C pyrolysis) exhibits doubled nitrate adsorption capacity ($175 \rightarrow 360 \text{ mg/g}$) via synergistic electrostatic interactions and ion exchange, particularly effective in acidic environments [35].

Alternative modification techniques effectively augment biochar's pore volume and specific surface area, creating abundant adsorption sites to facilitate denitrification processes. The synthesized biochar from desiccated yellow flag iris (*Iris pseudacorus*) through conventional pyrolysis under an inert atmosphere revealed heterogeneous surface morphology and hierarchical porosity conducive to microbial colonization. Iron amendment in this system demonstrated 14.7% improvement in total nitrogen (TN) removal efficiency compared to biomass-supplemented configurations [36]. Similarly, the developed iron-modified biochar (FeB) via FeCl_3 impregnation of bamboo-derived carbon for constructed wetland applications. Comparative analysis showed FeB's enhanced pore connectivity and surface roughness significantly increased microbial attachment density, thereby improving nitrogen transformation efficiency through optimized biofilm development [37].

The nano-MgO-sheet-incorporated biochar composites were successfully synthesized through MgCl_2 solution impregnation of diverse biomass feedstocks followed by pyrolysis at 600 °C. The MgO nanosheets demonstrated uniform morphology and homogeneous dispersion within the biochar matrix. These nanostructures exhibited effective nitrate adsorption via electrostatic interactions and ion exchange mechanisms, attributed to their surface positive charges in aqueous environments. High-resolution transmission electron microscopy (HR-TEM) analysis confirmed the biochar matrix's mesoporous structure with an average pore diameter of 50 nm. All MgO–biochar nanocomposites displayed superior phosphate (maximum 835 mg/g) and nitrate (maximum 95 mg/g) removal capacities,

particularly those derived from sugar beet tailings and peanut shells, outperforming conventional adsorbents [38]. Comparatively, magnetic biochar (MBC) was developed by loading α -Fe₂O₃ and Fe₃O₄ onto Douglas-fir-derived biochar (pyrolyzed at 900 °C, specific surface area: 494 m²/g). The MBC demonstrated rapid nitrate (15 mg/g) and fluoride (9 mg/g) removal through electrostatic interactions rather than Fe-F chemical bond formation, as evidenced by adsorption mechanism analysis [39].

3.2. Functional Group Regulation

Native biochar exhibits limited oxygen-containing functional groups (C–O, C=O, and –OH) on its surface [40]. Targeted modification strategies enable precise regulation of functional group composition, particularly enhancing –OH, –COOH, and C=O moieties. These engineered surface functionalities significantly modulate biochar's redox properties, thereby exerting critical influence on electron transfer dynamics during denitrification processes [41]. Acid–base treatment emerges as a predominant approach for oxygen–functional group incorporation. The implemented nitrogen-doping modification using urea, melamine, pyrrole, and pyridine effectively enhances nitrogen content and oxygen–functional group density on activated carbon surfaces. The pyrrole/pyridine treatments induced structural disordering in graphitic crystallites, generating abundant active sites that significantly improved denitrification efficiency [42]. HCl-mediated modification has demonstrated superior nitrogen removal capacity in constructed wetlands treating wastewater effluent compared to unmodified controls [43]. The developed iron-clad biochar (ICB) through sequential HCl/FeCl₃ treatment achieved enhanced specific surface area and hierarchical porosity alongside enriched surface hydroxyl groups. These structural modifications substantially improved ammonium adsorption capacity, with ICB exhibiting greater maximum ammonium uptake than raw biochar (RHB) due to optimized surface chemistry and pore architecture [44].

Beyond conventional acid/base modifications, researchers have incorporated transition metals to generate redox-active functional groups on biochar surfaces, significantly improving denitrification performance. Mn-modified nitrogen-enriched biochar catalysts for low-temperature NH₃-selective catalytic reduction were developed. The modified biochar exhibited dual Brønsted and Lewis acid sites, enhancing NH₃ adsorption capacity, while amorphous MnO₂ and pyrrolic N served as primary active centers to boost denitrification efficiency [45]. The magnetite-loaded rice husk biochar (MRHB) demonstrated enhanced heterotrophic denitrification under low C/N ratios. MRHB increased porosity and functional group density while facilitating electron transfer through iron-oxide-mediated pathways, achieving higher nitrate removal efficiency compared to unmodified controls [46]. These advancements highlight the strategic potential of metal–biochar composites in optimizing denitrification systems.

4. Mechanism of Biochar-Enhanced Denitrification

Microbial denitrification (nitrate respiration) involves intricate electron transport mechanisms (Figure 3) wherein NADH donates electrons to NADH dehydrogenase Complex I (NDH–1), which subsequently transfers electrons to coenzyme Q (CoQ). From CoQ, bifurcated electron transfer pathways emerge: (1) CoQ delivers two electrons to nitrate reductase (NaR), reducing nitrate (NO₃[–]) to nitrite (NO₂[–]); (2) CoQ sequentially transfers two electrons to Cytbc1 Complex III, which relays electrons to cytochrome c (Cyt c). Cyt c then mediates electron delivery through a reductase cascade, progressively reducing NO₂ to nitric oxide (NO), nitrous oxide (N₂O), and ultimately molecular nitrogen (N₂). Concurrent organic carbon oxidation to CO₂ drives ATP synthesis via chemiosmotic coupling [47,48]. Core enzymatic components governing this process include NaR, nitrite reductase (NiR),

nitric oxide reductase (NoR), and nitrous oxide reductase (NoS) [49,50]. Perturbations in enzymatic activity (e.g., competitive inhibition) or electron transport continuity (e.g., cofactor deficiency) directly impair denitrification efficiency. Biochar enhances this process through three synergistic mechanisms.

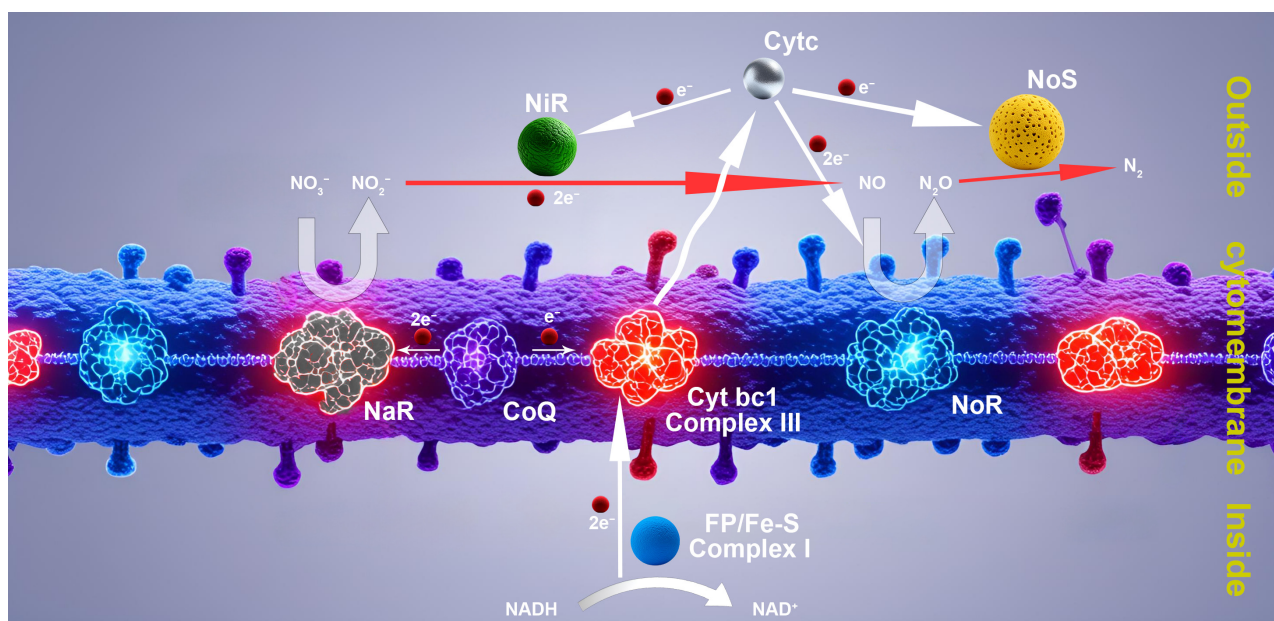


Figure 3. Electron transport chain of denitrification.

4.1. Enhancement of the Enzyme Activity and Functional Gene Expression

Denitrification is fundamentally driven by a cascade of metalloenzymes—NaR, NiR, NoR, and NoS—whose activity is strictly governed by the expression of functional genes such as *narG*, *nirK/S*, and *nosZ*. Biochar amendment has been demonstrated to significantly intensify this biochemical process by simultaneously upregulating these functional genes and enhancing the catalytic activity of their corresponding enzymes [49]. For instance, bamboo-derived biochar promotes the expression of denitrification genes (*napA*, *nirK*) alongside electron-transport-associated genes (*napB*, *napC*), thereby optimizing the electron transfer efficiency essential for enzymatic reactions [51]. Crucially, recent studies indicate that biochar upregulates *nosZ* expression and increases the copy numbers of NiR and NoS genes, which directly enhances the synthesis of the NoS enzyme responsible for the terminal reduction step ($\text{N}_2\text{O} \rightarrow \text{N}_2$) [52,53]. This regulatory function is particularly vital for mitigating nitrous oxide accumulation, as evidenced by low-temperature biochar applications where elevated NaR, NiR, and NoS activities were positively correlated with superior total nitrogen removal [54]. Collectively, these biochemical enhancements are attributed to biochar providing a stable microenvironment that concentrates substrates near enzymatic active sites, ensuring a robust and complete denitrification cascade.

However, a critical distinction must be made between microbial population enrichment and direct enzymatic activation. The elevated copy numbers of these functional genes, typically detected via qPCR or metagenomic sequencing, primarily reflect enhanced microbial growth and the macroscopic enrichment of denitrifying populations. Biochar's highly porous architecture and its capacity for sustained organic carbon or electron release offer an optimized protective microenvironment (niche), thereby amplifying the total biomass of denitrifying taxa. Crucially, an increase in DNA-level gene abundance does not inherently equate to an upgraded per capita electron transfer efficiency or enhanced intracellular enzymatic activity.

A major methodological limitation in contemporary literature is the near-exclusive reliance on DNA-based profiling. The vast majority of cited studies lack transcriptomic confirmation (RNA-level expression), specific enzyme activity assays, or real-time electrochemical electron-flux tracking. Therefore, the observed correlations between biochar addition and gene abundance should be interpreted as a reflection of expanded microbial carrying capacity and structural community shifts, rather than direct bioenergetic or thermodynamic acceleration of the electron transport chain.

4.2. Redox-Mediated Electron Transfer Pathways

Biochar-derived redox-active moieties, formed during pyrolysis, serve as effective “electron shuttles” (or redox mediators) that significantly catalyze pollutant biodegradation [24,55,56]. This functionality primarily originates from surface-abundant quinone functional groups, alongside the synergistic participation of O–H and C=O moieties, which act as critical components for reversible electron transfer [13,57,58]. As illustrated in Figure 4, the quinone-mediated denitrification enhancement operates through a two-phase redox cycle: initially, anaerobic bacteria transfer respiratory electrons to biochar’s quinonic moieties via dehydrogenase-catalyzed enzymatic reduction; subsequently, the reduced mediators abiotically transfer these electrons to nitrogen oxides, thereby facilitating contaminant reduction through non-biological pathways. This mechanism significantly reduces electron transfer resistance and accelerates denitrification kinetics. For instance, Fan et al. [36] demonstrated that quinone/phenolic groups in plant-derived biochar effectively mediate electron transfer in iron-based constructed wetlands, facilitating Fe(III) reduction through sustained electron shuttling to ultimately enhance iron-mediated autotrophic denitrification and overall nitrogen removal performance.

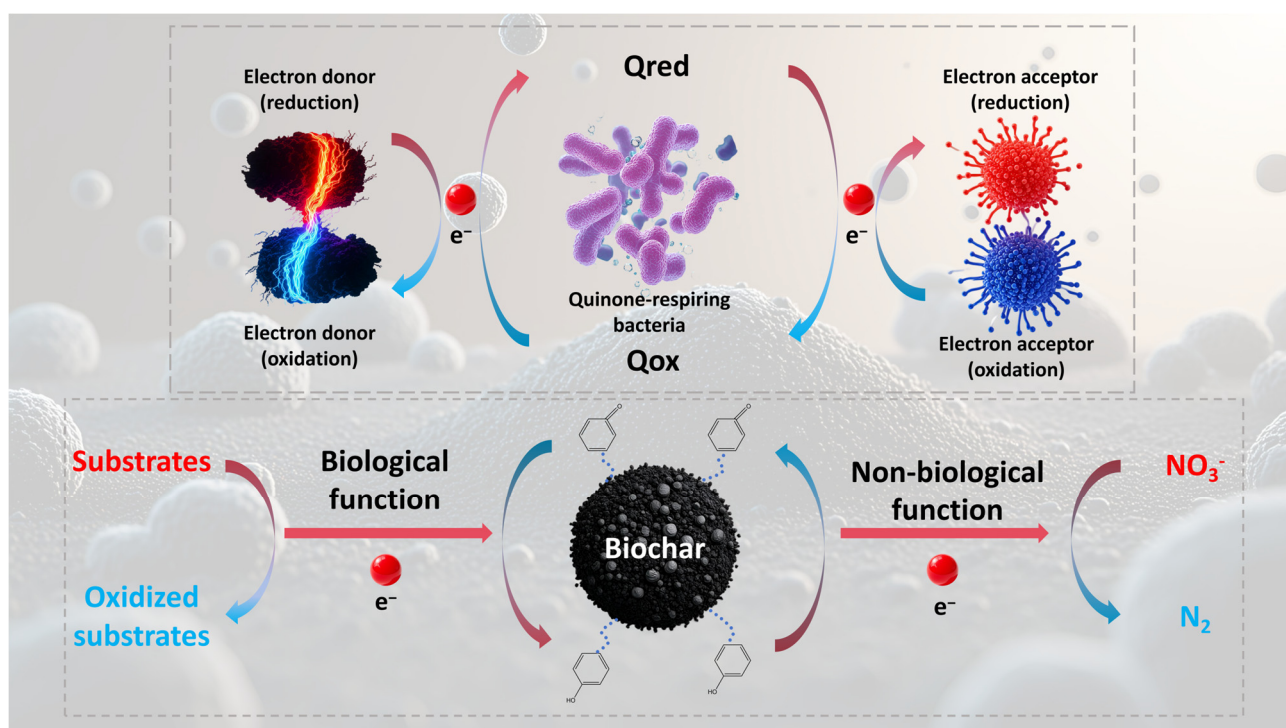


Figure 4. Mechanism of Action of the Biochar Oxygen Reduction Mediator.

4.3. Decoupling the Mechanistic Framework of Biochar-Mediated Electron Transfer

To avoid mechanistic ambiguity, a strict line must be drawn between electron shuttling, conductive matrix transfer, and true biological DIET (Table 3).

Table 3. Decoupling and comparison of electron transfer mechanisms in biochar-amended denitrification systems.

Comparison Dimensions/Characteristics	Electron Shuttling (MET)	Conductive	True DIET
Core Mechanism	Chemical/Functional-group-driven: Utilizes reversible redox-active functional groups (e.g., quinone/hydroquinone structures) on the biochar surface as transient “electron buffers” to accept and donate electrons.	Solid-state-physics-driven: Utilizes the highly developed conjugated π -electron network (graphitic structures) of high-temperature biochar to passively transmit electrons as microscopic “solid wires.”	Biological/Electrochemical codriven: Electroactive microbial consortia establish direct electrical continuity between cells using biochar as an external conductive medium or bridging conduit.
Definitive Proofs Required	1. Cyclic voltammetry (CV) exhibits distinct redox peaks characteristic of surface functional groups; 2. Chemical titration quantifies a substantial contribution of electron-accepting/donating capacities (EAC/EDC).	1. Determination of high solid-state electrical conductivity of biochar via the four-probe method; 2. Electrochemical impedance spectroscopy (EIS) reveals a significant reduction in charge transfer resistance within the system.	Generally requires high pyrolysis temperatures or specific engineering (e.g., heteroatom doping, transition metal loading) to satisfy cross-cellular conductive demands.
Pyrolysis Temperature	Low-to-medium temperature (typically 300–500 °C), which maximizes the retention of surface redox-active functional groups [54,59].	High temperature (typically ≥ 600 °C) to promote dehydrogenation, deoxygenation, and the graphitization of the carbonaceous skeleton [54,59].	Generally requires high pyrolysis temperatures or specific engineering (e.g., heteroatom doping, transition metal loading) to satisfy cross-cellular conductive demands.
Representative Empirical Evidence	Wu et al. [54] verified that 300 °C and 500 °C biochars are rich in surface redox-active functional groups, serving as excellent electron shuttles; Zhao et al. [59] demonstrated that 300 °C biochar facilitates functional-group-mediated electron shuttling to accelerate pyrite bio-oxidation and enhance electron supply; Yuan et al. [60] investigated the mechanism of loaded redox-active Fe acting as highly efficient electron shuttles to mitigate soil N ₂ O emissions by promoting complete denitrification.	Cayuela et al. [59] evaluated 22 biochars, demonstrating that high pyrolysis temperatures shift the dominant mechanism from surface electron buffering to solid-state matrix conduction; Wu et al. [54] comparatively showed that, at a high pyrolysis temperature of 800 °C, surface functional groups largely vanish, and electron transfer relies primarily on the highly graphitized carbon skeleton.	Li et al. [61] discovered in a single-stage partial denitrification/anaerobic ammonium oxidation (PD/A) system that biochar enriched specific functional genera (e.g., <i>Thauera</i> and <i>Candidatus Kuenenia</i>) and accelerated extracellular electron transfer (EET) by stimulating EPS secretion. This represents the cutting-edge frontier closest to the DIET boundary at the community level (though macroeconomic EET acceleration does not fully equate to true microscopic cell-to-cell pili/cytochrome bridging).

The mediated electron transfer (MET) pathway is dominated by the chemical redox-active functional groups (RAFTs) on the biochar surface. The reversible transformation between quinone and hydroquinone states allows biochar to serve as a transient electron sink or source. This process is chemical contact dependent but does not require bulk solid-state electrical conductivity or cell–cell aggregation.

Conductive electron transfer (CET) relies on the solid-state physics of the carbon matrix. High-temperature pyrolysis (>600 °C) expands the degree of graphitization, creating dense, continuous π -electron clouds. The biochar matrix acts purely as an electrical conduit or a macroscopic electrode that shunts electrons across distances.

True biological DIET via biochar is fundamentally a biological phenomenon requiring dedicated cellular architecture. In a biochar-assisted DIET scenario, the material acts as a conductive bridge between an electron-donating cell and an electron-accepting denitrifier. Critically, validating true DIET demands direct evidence of electroactive biological components, such as the upregulation of genes encoding outer-membrane cytochromes (OMCs, e.g., *omcS*, *omcZ*) or electrically conductive pili (e-pili, e.g., *pilA*), alongside direct cell-to-carbon microscopic aggregation.

We must note that a vast majority of current biochar denitrification studies casually claim the occurrence of “DIET” based solely on enhanced total nitrogen removal or CV profiles of the bulk mixed liquor. These parameters demonstrate enhanced EET activity but do not constitute definitive proof of true DIET. Future research must utilize transcriptomic analysis and advanced electrochemical microscopy to rigorously confirm cellular-level DIET mechanisms rather than conflating them with matrix conduction or surface shuttling.

4.4. Carbon Donor Effects from Genuine Redox-Mediated Enhancement

A cross-examination of contemporary literature reveals that biochar enhances denitrification via four intertwined pillars: adsorption sequestration, carbon source supply (DOC release), microbial community enrichment, and redox mediation. However, these mechanisms do not contribute equally; their relative importance shifts dynamically depending on pyrolysis temperature and influent carbon-to-nitrogen (C/N) ratios. Therefore, we systematically review the functions of these mechanisms and evaluate the relative weight matrix governing the contributions of distinct biochar types (Table 4).

Table 4. Relative importance and shifting weights of biochar mechanisms under different conditions.

Initial Conditions	Carbon Supply/DOC Release	Genuine Redox Mediation	CET	Physical Adsorption	Microbial Community Enrichment
Low-Temperature Biochar (300–450 °C) + Low C/N Wastewater	★★★★★ (Primary driver) [54,59]	★★ (Overshadowed by DOC leaching) [54]	★ (Lack of graphitic structure)	★★ (Small specific surface area)	★★★★ (Enrichment of heterotrophic denitrifiers) [62]
High-Temperature Biochar (≥600 °C) + Adequate C/N Wastewater	✗ (Negligible/Near zero) [54,59]	★★ (Loss of surface oxygen groups) [54]	★★★★★ (Primary driver) [54,63]	★★★★★ (High porosity favors mass transfer)	★★★★★ (Enrichment of electroactive/attached taxa) [63]
Fe/Mn-Modified Biochar Composite + Complex Wastewater System	★ (Depends on basal matrix pyrolysis)	★★★★★ (Driven by Fe ²⁺ /Fe ³⁺ redox couples) [59,60]	★★★★ (Metals enhance electron transport) [61]	★★★★★ (Abundant surface active sites) [60]	★★★★★ (Enrichment of metal-tolerant functional bacteria)

Note: The number of stars (★) semiquantitatively represents the relative contribution/importance of each specific mechanism under the designated scenario; ✗ indicates that the effect is negligible or undetected.

4.4.1. Low-Pyrolysis Biochars: Dominance of Carbon Donor Effects (DOC Release)

For low-temperature biochars (typically synthesized at 300–450 °C), the enhancement of denitrification is predominantly driven by carbon donor effects rather than active electron shuttling. At lower temperatures, incomplete thermal decomposition leaves large fractions of labile, aliphatic, and low-molecular-weight organic matter within the carbonaceous skeleton. When deployed in wastewater systems, these biochars undergo sustained leaching of DOC. This released DOC acts as an immediate, readily biodegradable electron donor that directly fuels heterotrophic denitrifiers, effectively mitigating carbon limitations in low-C/N domestic or industrial wastewater. While low-temperature biochars do possess abundant redox-active functional groups (e.g., phenolic and carboxylic groups), empirical mass balance studies indicate that the metabolic stimulation provided by leached DOC contributes up to 60–80% of the initial denitrification rate increase, overshadowing the concurrent electron-shuttling effects.

4.4.2. High-Pyrolysis and Modified Biochars: Dominance of Genuine Redox Mediation

Conversely, as the pyrolysis temperature escalates (>600 °C), the mechanism transitions abruptly toward genuine redox-mediated enhancement or conductive transfer. High-temperature pyrolysis triggers intense volatilization, drastically reducing the leachable DOC content to near-zero levels. Consequently, the carbon donor effect becomes negligible. Instead, the denitrification acceleration is driven by: (i) the large specific surface area which maximizes the physical adsorption of nitrate/nitrite and toxic intermediates (N₂O), (ii) the formation of dense, conductive graphitic π -electron channels, and (iii) the selective enrichment of electroactive, halophilic, or autotrophic denitrifying taxa.

4.4.3. Conditional Hierarchy of the Four Mechanisms

The relative importance and dominant hierarchy among these four mechanisms are not static but are highly contingent upon the specific combinations of biochar characteristics and environmental boundaries. When low-temperature biochar is deployed to treat carbon-deficient wastewater characterized by a low carbon-to-nitrogen ratio, the system dynamics are governed by a distinct hierarchical order where carbon source supply via dissolved organic carbon release acts as the paramount driver, followed sequentially by microbial enrichment, physical adsorption, and surface redox mediation. Under this specific operational configuration, the biochar functions fundamentally as a slow-release heterotrophic carbon pellet rather than an active electronic catalyst, meaning that the biological stimulation from the unlocked carbon source largely overshadows the synchronous functional-group-driven electron-shuttling effects.

Conversely, this mechanistic hierarchy undergoes a fundamental inversion when the operational scenario shifts to adequate carbon-to-nitrogen ratios coupled with the application of high-temperature or highly engineered biochars. In this alternative matrix, because high-temperature pyrolysis or intensive pre-washing drastically eliminates the leachable fraction of organic matter, the carbon donor effect becomes entirely negligible. Instead, the acceleration of wastewater denitrification is exclusively dictated by genuine redox mediation or solid-state conductive matrix transfer, complemented by physical adsorption and the selective enrichment of electroactive microbial taxa. In such systems, the biochar acts strictly as a solid-state heterogeneous catalyst that shunts electron flux across macroscopic distances without disrupting the external carbon balance, thereby offering a precise engineering dial to optimize electron-limiting or autotrophic denitrification processes.

By distinguishing these boundaries, future engineered systems can strategically select biochar types: utilizing low-temperature biochars for carbon-deficient streams to harness

the carbon donor effect, while employing high-temperature/metal-loaded biochars for high-strength or electrobiochemical systems where genuine redox mediation is required.

4.5. Microbial Community Engineering and Niche Differentiation

Beyond chemical mediation, biochar fundamentally alters the microbial ecology of wastewater systems through its hierarchical pore structure (micro-, meso-, and macropores) [54,64]. This architecture provides a sheltered microhabitat that shields sensitive denitrifiers from hydraulic shear forces and predatory protozoa, while simultaneously facilitating “niche differentiation” to enhance TN removal [65]. Liang et al. [18] observed enhanced ammonia and total nitrogen removal efficiencies in constructed wetlands supplemented with biochar, concomitant with increased relative abundances of nitrogen-cycling genera including *Nitrosomonas*, *Nitrospira*, and *Pseudomonas*. Jia et al. [43] reported that iron-modified biochar significantly improved total nitrogen removal by creating preferential microbial habitats that facilitate ecological niche differentiation without competitive exclusion. Specifically, the biochar surface supports aerobic biofilms for nitrification, whereas oxygen-limited inner pores maintain anaerobic conditions for denitrification; this spatial heterogeneity enables simultaneous nitrification and denitrification (SND) within a single particle and prevents competitive exclusion [43]. Concomitant with this habitat optimization is the selective enrichment of nitrogen-cycling genera, including *Nitrosomonas*, *Nitrospira*, *Thauera*, *Paracoccus*, and *Pseudomonas* [18,66]. Notably, Proteobacteria—a phylum containing characteristic denitrifiers often possessing outer-membrane cytochromes for solid-phase electron transfer—consistently emerges as the dominant taxonomic group [65]. These collective findings confirm that biochar amendment modulates species richness and community diversity through microenvironment optimization, thereby driving enhanced nitrogen removal efficiency.

Biochar enhances denitrification efficiency through three principal mechanisms: (1) upregulation of key denitrifying enzymatic activities (nitrate reductase, nitrite reductase) and functional gene expression (*napA*, *nirK*) to potentiate nitrate/nitrite reduction capacity; (2) provision of porous matrices that increase microbial colonization surfaces, thereby enriching taxonomic diversity and abundance of denitrifying consortia; (3) surface redox-active quinone/phenolic moieties functioning as electron shuttles to accelerate interfacial electron transfer between microorganisms and terminal electron acceptors. These synergistic mechanisms position biochar as a multifunctional biomaterial with significant potential for advanced water treatment and environmental remediation applications.

At the molecular and enzymatic levels, the mechanisms by which biochar regulates microbial nitrogen transformations are intrinsically linked to its ability to modulate functional gene abundances and enhance specific enzymatic activities. Biochar amendment typically alters the transcription and expression of key denitrification genes responsible for successive nitrogen reduction steps. For instance, the labile carbon fractions and high aliphaticity of pristine manure-derived biochars have been shown to supply advanced EDC, directly upregulating the absolute and relative abundances of *nosZ* genes (both clades I and II) and increasing the *nosZ*/(*nirK* + *nirS*) ratio [8]. This stimulation of complete denitrification pathways effectively mitigates gaseous nitrogen loss by accelerating the reduction of N_2O to N_2 in diverse ecosystems, including subtropical tea plantations [6]. Furthermore, when paired with organic matrices like digestate in paddy soils, biochar significantly upregulates *nosZ* expression to drive completely coupled denitrification and AOM processes [9]. Concurrently, biochar optimizes microbial community assembly to balance upstream gene abundances (such as *narG*, *nirS*, and *nirK*), thereby preventing the toxic accumulation of intermediates like NO_2^- and NO [6,8].

Beyond genomic modulation, biochar serves as a powerful catalyst that alters intracellular and extracellular enzymatic velocities. Sewage-sludge-derived biochars have been reported to selectively screen specific denitrifying strains, thereby promoting the synthesis and metabolic efficiency of nitrate and nitrite reductases (NAR and NIR) [11]. For lignocellulose-rich feedstocks like cornstalks, the surface-bound phenolic and quinone functional groups act as redox-active centers to stimulate complex carbohydrate hydrolysis, markedly promoting cellulase activity and cellulose degradation rates to sustain a continuous supply of bioavailable carbon for heterotrophic denitrifiers [7]. Crucially, the conductive carbon skeleton of biochar can function as a solid electron shuttle or “geoconductor,” speeding up the microbial electron transport chain. This is evidenced by markedly elevated system-wide electron transport system activity (ETSA) and dehydrogenase activities within biofilm carriers [10,11]. To systematically delineate these microscopic regulatory behaviors before exploring their macroscopic engineering implementations, the specific response characteristics of functional genes and enzymes under biochar induction are detailed in Table 5.

Table 5. Response characteristics of functional genes and enzymatic activities during biochar-regulated microbial denitrification.

Target Gene/Enzyme	Catalytic Step/Role	Biochar-Induced Regulatory Response	Mediating Mechanisms (How Biochar Works)	References
<i>nosZ</i> Gene (I/II)	$\text{N}_2\text{O} \rightarrow \text{N}_2$ (nitrous oxide reduction)	Significantly upregulated absolute/relative abundance; increased <i>nosZ</i> /(<i>nirK</i> + <i>nirS</i>) ratio.	The DOC and aliphatic carbon fractions of pristine biochar provide advanced electron-donating capacity, driving complete denitrification.	[8,9]
<i>narG</i> / <i>nirS</i> / <i>nirK</i>	$\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO}$ (upstream denitrification steps)	Gene abundances stimulated; better balanced under biochar amendment.	Alleviated toxic intermediate accumulations (NO_2^- and NO) by optimizing microbial community assembly.	[6,8]
NAR and NIR	Nitrate and nitrite reductase activities	Enhanced intracellular/extracellular specific enzymatic velocities.	Surface structures of sludge-derived biochar screen specific denitrifying strains and boost corresponding enzyme synthesis.	[11]
Cellulase Enzyme	Carbon source bioavailability	Enzymatic activity and cellulose degradation rate significantly promoted.	Surface-bound phenolic/quinone groups serve as redox-active centers to stimulate compound enzyme degradation.	[7]
ETSA	Electron transport system activity	System-wide ETSA and specific dehydrogenase activities markedly increased.	Biochar functions as an effective geoconductor and solid electron shuttle, speeding up the electron transfer chain.	[10,11]

Note: NAR, nitrate reductase; NIR, nitrite reductase; ETSA, electron transport system activity; DOC, dissolved organic carbon.

4.6. Classification and Comparative Review of Biochar Roles Across Diverse Nitrogen Removal Systems

To avoid mechanistic overgeneralization, it is imperative to decouple the distinct biophysical roles and electron transfer pathways of biochar across different biological nitrogen removal (BNR) configurations (Figure 5 and Table 6). Although biochar consistently serves as a performance booster, its precise catalytic behavior dictates fundamentally disparate metabolic flows depending on whether the system operates under heterotrophic, autotrophic, anaerobic, or aerobic pathways.

Mechanisms of Biochar-Enhanced Denitrification Across Diverse BNR Systems

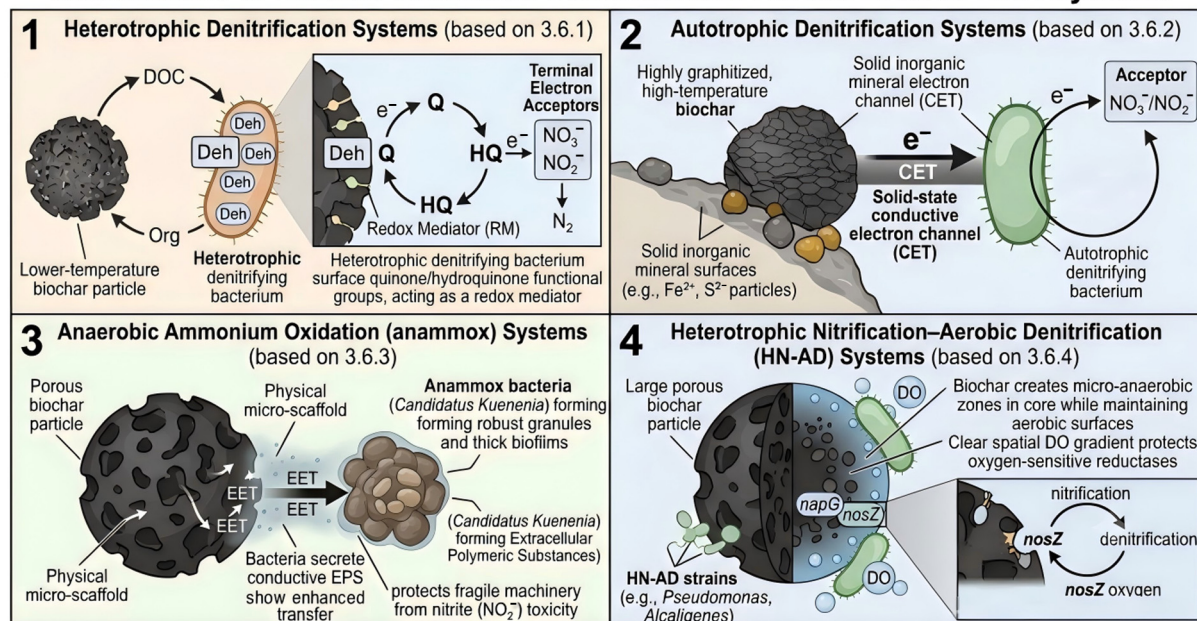


Figure 5. Mechanisms of biochar-enhanced denitrification across diverse BNR systems.

Table 6. Comparative matrix of biochar’s functional roles and electron transfer pathways across four major nitrogen removal systems.

Nitrogen Removal System	Primary Electron Donors and Acceptors	Dominant Functional Role of Biochar	Key Electron Transfer Pathway	Representative Functional Microbes	Major System Bottlenecks
Heterotrophic Denitrification	Donor: Leached DOC/external organics Acceptor: NO_3^- , NO_2^- , N_2O	Slow-release carbon source; redox mediator (surface functional groups)	Organics $\rightarrow$ Cyt c $\rightarrow$ Biochar RAFGs $\rightarrow$ Denitrifying reductases	<i>Thauera</i> , <i>Paracoccus</i> , <i>Pseudomonas</i> [54,67]	Rapid depletion of labile DOC; secondary organic pollution risks
Autotrophic Denitrification	Donor: Fe(II), sulfide, pyrite, H_2 Acceptor: NO_3^- , NO_2^-	Solid-state CET; mineral–microbe bridge	Inorganic Minerals $\rightarrow$ Biochar graphitic lattice $\rightarrow$ Autotrophic taxa	<i>Thiobacillus</i> , <i>Gallionella</i> , <i>Sulfurimonas</i> [68]	Surface passivation via dielectric mineral scaling (CaCO_3 , oxides)
Anaerobic Ammonium Oxidation (Anammox)	Donor: NH_4^+ Acceptor: NO_2^-	Physical micro-scaffold; EPS secretion stimulant; cell aggregator	Intracellular redox cycling; accelerated EET via biochar-stimulated EPS	<i>Candidatus Kuenenia</i> , <i>Candidatus Brocadia</i> [61]	Extremely slow bacterial growth; long start-up periods
Heterotrophic Nitrification–Aerobic Denitrification (HN-AD)	Donor: Organic carbon Acceptor: O_2 (nitrification); $\text{NO}_3^-/\text{NO}_2^-$ (denitrification)	DO gradient buffer; interfacial microaerobic niche provider	Dual pathways: Aerobic oxidation paired with pore-shielded anaerobic reduction	<i>Pseudomonas stutzeri</i> , <i>Alcaligenes</i> [62]	Intense competition for active sites between oxygen and nitrogen oxides

Note: RAFGs = Redox-Active Functional Groups; CET = Conductive Electron Transfer; EET = Extracellular Electron Transfer; DO = Dissolved Oxygen.

4.6.1. Heterotrophic Denitrification Systems

In traditional heterotrophic denitrification, biochar primarily functions under a dual regime: as a continuous carbon donor (via DOC leaching at low pyrolysis temperatures) and as a redox mediator (via surface quinone/hydroquinone functional groups). The electron flux originates from external or biochar-derived organic matter, moves through intracellular dehydrogenases, and is shunted via biochar’s surface functional groups to terminal electron acceptors (NO_3^- and NO_2^-), accelerating the rate-limiting steps of nitrogen reduction.

4.6.2. Autotrophic Denitrification Systems

Conversely, in autotrophic denitrification (e.g., driven by sulfur, pyrite, or hydrogen), biochar transitions strictly into a solid-state CET or inorganic catalyst. Because real organic carbon sources are omitted, the electron flux originates from solid inorganic minerals (such as Fe^{2+} or S^{2-}). High-temperature, highly graphitized biochar acts as a microscopic wire, extracting electrons from mineral surfaces and directly transferring them to autotrophic denitrifiers, thereby circumventing the sluggish mass transfer limitations inherent in pure mineral–microbe contact.

4.6.3. Anaerobic Ammonium Oxidation (Anammox) Systems

Within anammox consortia, the role of biochar shifts predominantly toward a physical micro scaffold for cell aggregation and an EPS stimulant. The slow-growing anammox functional bacteria (*Candidatus Kuenenia*) rely on biochar's porous matrices to self-aggregate into robust granules or thick biofilms. Chemically, biochar stimulates the secretion of conductive EPSs, which dramatically enhances macroscopic EET within the anaerobic bioreactor, protecting the fragile anammox machinery from nitrite toxicity and environmental shocks.

4.6.4. Heterotrophic Nitrification–Aerobic Denitrification (HN–AD) Systems

In HN–AD configurations, biochar serves as an aerobic oxygen buffer and protective niche. Unlike the strictly anaerobic pathways mentioned above, HN–AD strains (e.g., *Pseudomonas* or *Alcaligenes*) operate under high dissolved oxygen (DO) tensions. Biochar's rich macropore structure creates micro anaerobic zones within its internal core while maintaining aerobic surfaces. This unique spatial DO gradient shields oxygen-sensitive reductases (such as *napG* or *nosZ*) from bulk oxygen inhibition, allowing simultaneous nitrification and denitrification to proceed within a single, aerated microenvironment.

5. Application of Biochar in Denitrification

Current strategies leveraging biochar's dual functionality in denitrification involve direct stimulation through porous structure-mediated nitrate adsorption, surface-functional-group-enabled electron acceptor provision, and microbial activity enhancement, achieving efficient nitrogen transformation via optimized denitrification pathways. Advanced methodologies further exploit biochar's superior carrier capacity through immobilization of nanoscale zero-valent iron (nZVI) or copper ions to catalyze reductive reactions, coupled with synergistic microbial partnerships (e.g., denitrifier consortia), demonstrating enhanced nitrogen removal efficiency and system stability through combined biochar–metal–microbe interactions.

5.1. Direct Denitrification with Biochar

Biochar directly improves denitrification efficiency through multiple mechanisms, including the provision of electron donors to support microbial reduction reactions, environmental conditioning via pH and temperature modulation, and enhancement of denitrifying enzyme activity to accelerate nitrate conversion. Specifically, Berger et al. [69] demonstrated that biochar addition under intense rainfall conditions significantly enhanced nitrogen removal performance in woodchip biofilters, with substantially improved nitrate removal rates attributed to biochar-induced system stabilization. Further studies indicate that biochar effectively promotes overall denitrification efficacy by altering pore water retention to prolong hydraulic residence time, reducing dissolved oxygen concentrations to establish anaerobic microenvironments, and increasing microbial biomass to enrich denitrifying bacterial communities.

Biochar produced at different pyrolysis temperatures exhibits significant variations in nitrate reduction efficiency, primarily attributed to pyrolysis-dependent alterations in redox activity. Structural and chemical composition changes induced by thermal gradients result in distinct redox-active components during microbial denitrification processes. Specifically, low-temperature biochar (300–400 °C) is dominated by electron-donating phenolic compounds, medium-temperature derivatives (500–600 °C) predominantly contain electron-accepting quinones, while high-temperature variants (700–800 °C) develop condensed aromatic hydrocarbon structures [24], with these temperature-dependent physicochemical properties systematically summarized in Table 7.

Table 7. Redox components produced by biochar at different temperatures.

Pyrolysis Conditions	Redox Components	Characteristic	References
Low-temperature heat treatment	Phenolic group OH	The reduced phenolic components in low-temperature pyrolytic biochar were oxidized to provide electrons to promote the reduction of N ₂ O.	[24,25,54]
Medium-temperature heat treatment	Benzoquinonyl C=O	Microorganisms transfer electrons from organic matter to quinones, which are reduced to hydroquinone, which is then oxidized to quinones and transferred to nitrates.	[13,24]
High-temperature heat treatment	Conjugated aromatic hydrocarbons	The conductive structure of condensed aromatic hydrocarbons was used as an alternative electron acceptor to reduce the total nitrogen denitrification.	[24,25]

Sathishkumar et al. [13] demonstrated that biochar synthesized across pyrolysis temperatures (300–800 °C) universally enhances microbial nitrate reduction, with biochar derived at 500 °C (BC500) exhibiting superior electron transfer capacity. Surface characterization revealed BC500's phenolic hydroxyl and quinone carbonyl groups mediate redox shuttling, accelerating nitrate-to-nitrite conversion kinetics. Chen et al. [25] delineated temperature-specific functions: 300 °C biochar improves intermediate product management through pore-assisted metabolite retention, while 800 °C biochar promotes N₂O-to-N₂ conversion via graphitic domain-enhanced electron conduction. Wu et al. [54] systematically investigated biochar pyrolyzed at 300 °C, 500 °C, and 800 °C, demonstrating that biochar derived at 300 °C not only enhanced denitrifying enzyme activity (particularly nitrate reductase and nitrite reductase) but also significantly reduced N₂O accumulation through optimized electron allocation. Concurrently, this biochar variant improved electron transport system activity, collectively achieving substantial nitrate removal efficiency. Furthermore, 300 °C biochar increased the abundance of denitrifying microbial communities, with pronounced enrichment of *Pseudomonas* populations, whose metabolic specialization in complete denitrification pathways (NO₃[−] → N₂) was observed to strengthen via microbial community restructuring. This functional microbial selection mechanism ultimately optimizes nitrogen removal through synergistic biochar–microbe interactions.

Comparative studies have revealed that biochar synthesized under low-temperature pyrolysis conditions retains significantly higher concentrations of oxygen-containing surface functional groups (e.g., hydroxyl, carboxyl, phenolic hydroxyl) compared to high-temperature counterparts. These functional moieties not only enhance the material's ion-exchange capacity and adsorption capabilities but more critically mediate the sustained release of DOC, which functions as both a continuous carbon substrate and electron donor. This controlled DOC release mechanism effectively stimulates the metabolic activity of

denitrifying microbial communities through enhanced electron shuttle systems and cofactor regeneration, thereby significantly improving nitrate reduction efficiency in engineered remediation systems. Jia et al. [70] systematically investigated a low-temperature pyrolyzed corncob biochar (CCB)-integrated stormwater bioretention system, demonstrating that the pyrolysis process confers CCB with high aromaticity and hydrophobicity. These structural characteristics significantly prolong the release of soluble organic compounds from the biochar matrix, enabling sustained provision of essential carbon substrates for denitrifying microbial communities within the system.

Further investigations by Jiang's group [71] have systematically elucidated the efficacy and mechanisms of low-temperature pyrolyzed coconut shell biochar in enhancing concurrent nitrogen and phosphorus removal within biological treatment systems. These studies reveal that the oxygen-containing functional groups (e.g., carboxyl, lactone) and highly ordered aromatic carbon matrix inherent in such biochar create optimal microenvironments for HN-AD bacteria. The identified functional groups not only participate directly in redox cycling but also act as electron mediators, effectively activating key metabolic enzymes (e.g., ammonia monooxygenase, nitrate reductase) in HN-AD bacteria, thereby accelerating the transformation and removal of nitrogen species ($\text{NH}_4^+ - \text{N}$, $\text{NO}_3^- - \text{N}$) and phosphorus. Furthermore, the material's large specific surface area and hierarchical porous architecture promote microbial adhesion, colonization, and biofilm development, substantially increasing the biomass and metabolic vigor of functional microorganisms. This structural advantage spatially optimizes the system's contaminant removal capacity through enhanced microbial-electrochemical interactions at the biochar-water interface.

Pyrolysis temperature emerges as a critical determinant governing biochar's physico-chemical characteristics. Biochar synthesized at varying pyrolysis temperatures exhibits significant differences in the typology, abundance, and stability of surface functional groups, which directly modulate its electron-donating/shuttling capacity and bidirectional regulation of denitrification-critical enzymatic activities (e.g., nitrate reductase stimulation vs. nitrite reductase inhibition). Concurrently, thermal gradients differentially control biochar's regulatory effects on microbial community structure, population dynamics, and metabolic vigor. The temperature-dependent surface chemistry (functional group evolution), enzyme activity modulation (enhancement/suppression dichotomy), and comprehensive microbial community management collectively constitute the mechanistic basis for the performance disparity of biochar in denitrification systems across thermal preparation regimes.

5.2. Biochar-Supported Metallic Nanomaterials

As a cost-effective carbon-rich material, biochar exhibits exceptional metal nanoparticle carrier capacity owing to its large specific surface area, porous architecture, and strong ion-exchange capabilities. The integration of nanomaterials (e.g., nanoscale zero-valent iron, NZVI) with biochar matrices enhances surface reactivity and electron transfer efficiency, thereby optimizing denitrification performance. Although NZVI demonstrates superior nitrate removal capacity, its practical application is constrained by aggregation propensity and oxidative instability. Wang et al. [72] demonstrated that biochar-supported nZVI (nZVI-BC) enhanced nitrogen removal efficiency (5.3–11.7%) and rate (5.2–11.5%) in anammox systems through selective enrichment of anammox bacteria and reinforced EET pathways. Chen et al. [63] established comparative anammox reactors using nZVI-modified biochar (nZVI@BC) versus conventional biochar (BC), revealing sustained nitrogen removal efficiency (90%) under low-temperature stress in nZVI@BC systems versus performance decline (74.4%) in BC controls. The nZVI@BC amendment promoted EPS synthesis, improved biomass granulation, and elevated anammox bacterial abundance. Li et al. [73] engineered a bimetallic Fe/Ni-biochar composite (BC@Fe/Ni) achieving 93% nitrate removal at concen-

trations < 50 mg/L without pH adjustment, with reaction kinetics demonstrating 1.75-fold enhancement over pristine nZVI, attributable to synergistic electron donation and catalytic activation mechanisms.

5.3. Biochar-Immobilized Microbial Consortia

Microbial biodegradation effectively resolves the critical limitation of saturated biochar adsorption by preventing pollutant re-release from exhausted biochar, thereby significantly enhancing its regeneration potential [51,62]. Biochar provides ideal habitats for denitrifying bacteria through abundant surface colonization sites, facilitating robust microbial proliferation while augmenting resilience against environmental stressors (e.g., pH fluctuations, toxic substance exposure) and metabolic activation, ultimately achieving efficient denitrification enhancement. Liu et al. [67] immobilized *Paracoccus* sp. YF1 on bamboo-derived biochar, demonstrating superior nitrate removal efficiency compared to free bacterial cells under varying environmental conditions (temperature, salinity). This performance stability stems from biochar's physical protective barrier against environmental perturbations. Notably, the immobilized *Paracoccus* sp. YF1 maintained 99.8% denitrification efficiency through ten operational cycles, demonstrating exceptional reusability. Yu et al. [62] identified significant synergies between modified walnut shell biochar and *Pseudomonas* XL-2 in ammonium removal, achieving 96.34–98.73% efficiency in SBRs with biochar–microbe composites, far exceeding free-cell systems. This synergy arises from biochar's structural–chemical characteristics: irregular pore architectures and hydrophilic functional groups (–OH, –COOH) provide stable immobilization sites, while enhanced mass transfer optimizes substrate accessibility to microbial cells, driving efficient nitrogen removal [74]. Mechanistic studies reveal biochar's large surface area and hierarchical pore networks (micro-/mesopores) increase reactive site density, improving substrate transport efficiency. Surface redox-active groups (phenolic hydroxyl, quinone) act as electron shuttles to accelerate electron transfer rates, while inherent pH buffering capacity maintains stable microenvironments for microbial activity. These multifunctional attributes collectively enable superior contaminant removal and system stability in aerobic denitrification systems, as evidenced by Mao [75].

5.4. Engineering Applicability and Long-Term Operational Bottlenecks

While laboratory-scale, short-term batch experiments using synthetic feeds have consistently verified the efficacy of biochar in accelerating denitrification, evaluating its operational longevity under realistic engineering boundaries remains an indispensable bottleneck. In large-scale, continuous-flow bioreactors, biochar undergoes intricate physicochemical aging and faces intense thermodynamic competition from complex real wastewater matrices, both of which can compromise long-term nitrogen removal stability.

5.4.1. Long-Term Material Aging and Electrochemical Decay Mechanisms

Over extended operational cycles, pristine or modified biochars inevitably suffer from performance decay driven by structural and electrochemical aging. First, the loss or passivation of RAFGs poses a major threat to electron-shuttling systems. Continuous redox cycling can trigger the irreversible overoxidation of quinone/hydroquinone moieties or the persistent leaching of surface-bound oxygen groups, narrowing the biochar's net EAC/EDC. Second, solid-state conductivity decay is frequently observed in conductive transfer systems, primarily caused by the structural deposition of dielectric minerals (e.g., calcium carbonate scaling) or the mechanical fracturing of the graphitic carbon skeleton under hydrodynamic shear [59]. Third, for low-temperature biochars, the rapid initial depletion of leachable DOC permanently strips the material of its “carbon donor effect,” shifting the biological burden entirely back to external carbon dosing [54]. Lastly, physical pore blockage restricts substrate mass transfer. Beyond inorganic precipitation, the excessive accumulation of aged EPSs and dead biomass

within the macro- and mesopores causes “biofilm fouling.” This severe pore clogging screens the active catalytic sites and electrically conductive pathways from coming into direct physical contact with nitrate/nitrite reductases [62].

5.4.2. The Inhibitory Interferences of Complex Wastewater Matrices

Beyond internal material degradation, engineered biochar denitrification systems must withstand the complex chemical matrices characteristic of real-world domestic and industrial streams. The presence of background dissolved organic matter (DOM) often triggers intense competitive adsorption, where high-molecular-weight humic substances or surfactants block surface active sites and physically displace nitrate molecules [67]. In sulfur-rich streams, coexisting sulfides act as aggressive alternative electron donors, inducing an unregulated “electron flux diversion” that disturbs the established syntrophic or autotrophic electron transfer networks. Furthermore, heavy metal accumulation (e.g., Cu^{2+} , Cd^{2+} , Pb^{2+}) within biochar pores exerts localized cellular toxicity, actively inactivating the metallic cofactors embedded within microbial *narG*, *nirK/nirS*, and *nosZ* enzymes [63]. Finally, severe salinity fluctuations—common in industrial saline wastewaters—induce violent osmotic shock in the attached biofilm consortia. High osmotic pressure drastically deforms the cell membrane structure of denitrifying bacteria, triggering the lysis of electroactive cohorts and decoupling the biological machinery from the biochar’s conductive graphitic matrices. Consequently, mitigating matrix interference through targeted material surface engineering (e.g., selective hydrophobic coating or specific catalytic loading) represents a critical pathway for the future commercialization of biochar-driven wastewater systems.

5.5. Practical Challenges and Engineering Limitations

Despite the promising results observed in laboratory-scale studies, the transition of biochar-enhanced denitrification from bench-scale to full-scale wastewater treatment plants (WWTPs) faces several critical engineering bottlenecks that warrant rigorous scrutiny.

First, the long-term stability and “aging effect” of biochar remain a primary concern. Most current studies operate on short-term cycles (weeks to months), failing to capture the inevitable degradation of biochar surfaces over years of operation [76]. The crucial electron-shuttling functional groups (e.g., quinone/hydroquinone moieties) are susceptible to oxidation and passivation by wastewater components. As the biochar “ages,” its redox-active capacity may decline, potentially transforming it from an active catalyst back into a mere physical support, thereby diminishing its cost-effectiveness over time.

Second, hydraulic risks, particularly clogging and flow channeling, pose operational hazards in fixed-bed reactors and constructed wetlands. While biochar’s hierarchical pore structure is beneficial for microbial colonization, the proliferation of excessive biofilm and the accumulation of suspended solids can lead to pore occlusion. This reduces the effective hydraulic conductivity, causing short circuiting and reducing the hydraulic residence time essential for complete denitrification. Furthermore, powdered biochar, despite its high reactivity, is difficult to recover and may wash out in effluent, necessitating the development of robust granular or composite formulations.

Third, the risk of secondary pollution cannot be overlooked. Depending on the feedstock (e.g., sewage sludge) and pyrolysis conditions, biochar may contain residual heavy metals or persistent organic pollutants (e.g., polycyclic aromatic hydrocarbons, PAHs). The potential leaching of these toxic substances, along with the initial release of excessive DOC which contributes to effluent COD spikes, requires strict quality control and pre-treatment protocols (e.g., washing or acid pickling) before large-scale deployment.

Finally, economic feasibility regarding scale-up production is often underestimated. While low-temperature pyrolysis saves energy, achieving the precise “structure–function”

tailoring required for high-efficiency denitrification often demands complex modification steps (e.g., metal doping, acid washing), which significantly inflates the material cost. Future technoeconomic analyses must weigh the denitrification gains against the life-cycle assessment (LCA) costs of manufacturing these advanced biochar composites.

6. Conclusions

Biochar has emerged as a revolutionary, cost-effective, and highly tunable carbonaceous platform for accelerating the rate-limiting steps of biological nitrogen removal in wastewater engineering. This comprehensive review systematically highlights that biochar's contribution transcends basic physical adsorption, acting primarily as a pivotal redox-active mediator that fundamentally reshapes electron transfer pathways during denitrification. The synthesis fingerprints, which are dictated by feedstock inheritance and precise pyrolysis kinetics, enable customized tailoring of the resulting surface area, pore architectures, and surface functional group profiles. These structural evolutions directly govern the material's solid-state electrochemical behavior, notably its electron-accepting and -donating capacities.

Mechanistically, the presence of biochar uplifts the biological denitrification system in multiple interconnected dimensions. Firstly, its hierarchical porosity provides stable cytocompatible scaffolds that protect and cultivate dense denitrifying biofilms, significantly shifting the microbial community structure toward highly functional electroactive cohorts. Additionally, biochar serves as an extracellular electron conduit that upregulates the transcription and copy numbers of core denitrification-critical genes, such as *narG*, *nirS*, *nirK*, and *nosZ*. Furthermore, the accelerated electron flux systematically boosts the biological activities of key respiratory reductases, ensuring rapid and complete nitrate-to-nitrogen gas conversion kinetics while simultaneously mitigating transient nitrous oxide accumulation.

7. Future Perspectives and Emerging Frontiers

To successfully transition biochar-augmented denitrification technologies from empirical laboratory benches to industrialized, large-scale reality, future research must transcend descriptive, qualitative summaries and actively converge with cutting-edge cross-disciplinary paradigms.

7.1. Deep Mechanistic Decoupling via Multiomics and Electron Flux Dynamics

Current mechanistic insights are heavily constrained by descriptive correlations based on traditional microbial profiling and baseline quantitative polymerase chain reaction frameworks. Future endeavors must deploy deep multiomics frameworks, specifically combining metatranscriptomics, metaproteomics, and high-throughput metabolomics, to precisely track real-time active metabolic pathways and protein synthesis within biochar-biofilm interfaces. Concurrently, the implementation of advanced in situ microelectrode techniques, electrochemical impedance spectroscopy, and scanning electrochemical microscopy is imperative to quantitatively measure live interfacial electron transport rates. These combined approaches will definitively resolve whether biochar serves as an active cellular metabolic electronic trigger or merely acts as a passive physical matrix that inflates macroscopic biomass yields.

7.2. Artificial-Intelligence-Assisted Optimization and Machine Learning

The enormous structural diversity of biomass feedstocks coupled with multivariable pyrolysis operations creates an overwhelmingly complex design space for material development. Future breakthroughs should leverage data-driven methodologies by integrating advanced machine learning architectures, such as extreme gradient boosting, random

forest, and deep neural networks. Large-scale empirical datasets can be utilized to train predictive machine learning models, enabling scientists to accurately forecast resulting biochar topographies and electrochemical capacities without exhaustive laboratory testing. Furthermore, interpretable causal machine learning frameworks can dynamically optimize operational wastewater variables, including dynamic carbon-to-nitrogen ratios, dissolved oxygen fluctuations, and temperature shifts, to maximize total nitrogen removal while mathematically minimizing carbon footprints.

7.3. Integration into IoT-Driven Smart Wastewater Treatment Systems

Moving beyond isolated reactor configurations, future engineered biochar frameworks should be seamlessly synthesized into Internet-of-Things-enabled smart wastewater treatment infrastructures. By constructing a high-fidelity digital twin of the bioreactor system, continuous real-time monitoring and rapid responses to industrial shock loadings can be achieved. Live streams of metadata from inline sensors tracking oxidation–reduction potential, pH, dissolved oxygen, and nitrate or nitrite fluctuations can be coupled with predictive artificial intelligence automated control loops. When facing unpredictable environmental stressors or low-temperature depressions, the smart system can automatically execute instant closed-loop interventions, such as micromodulating influent fluidization velocities, precisely titrating external carbon sources, or pulsing localized electrochemical currents, to sustain steady-state electron fluxes and protect microbial consortia.

7.4. Advanced Next-Generation Biochar Engineering Strategies

The material science of biochar must undergo a paradigm shift from traditional, crude bulk modifications toward precision, atomic-scale tailoring. Future strategies should prioritize the development of carbon-supported single-atom catalysts, such as iron, nitrogen, and carbon transition complexes, directly anchored onto biochar scaffolds. Isolating single metal atoms onto the graphitic matrix maximizes atomic utilization efficiency and successfully mimics the dense active sites of biological enzymes, thereby drastically lowering the activation energy required for enzyme-mediated electron transfers. Additionally, fabricating stimuli-responsive smart biochars presents a compelling frontier. These intelligent matrices can adaptively alter their surface charges, functional group exposures, or solid-state conductivities in response to real-time ambient environmental stimuli, such as salinity surges or localized pH shifts, creating intelligent microenvironmental niches tailored to biological demands.

7.5. Sustainability, Ecosafety, and Commercial Carrier Engineering

From an industrial application perspective, the life-cycle footprint and operational practicality of biochar systems require systemic refining across both material synthesis and engineering deployment. Regarding green pyrolysis engineering, novel carbonization technologies must be engineered to drastically lower pyrolysis temperature barriers and reduce energy consumption while establishing closed-loop capture systems to eliminate hazardous volatile organic compounds, tars, and fine particulate emissions generated during calcination. Meanwhile, advanced composite engineering must be refined to fabricate highly resilient immobilized carriers, such as biochar–sodium alginate microspheres or biochar–magnetic composites. These engineered configurations will minimize material washout, enhance matrix–pollutant mass transfer, and ensure convenient magnetic or physical separation, thereby cementing a robust foundation for long-term continuous wastewater treatment applications.

Author Contributions: Conceptualization, Y.W., S.X. and B.Y.; Methodology, S.L. and H.Z. (Haochun Zang); Software, L.W. and H.Z. (Haiya Zhang); Validation, Y.W., S.X. and B.Y.; Formal Analysis, S.L.; Investigation, H.Z. (Haochun Zang); Resources, B.Y.; Data Curation, Y.W. and H.Z. (Haochun Zang); Writing—Original Draft Preparation, Y.W.; Writing—Review and Editing, S.X. and B.Y.; Visualization, Y.W.; Supervision, S.X. and B.Y.; Project Administration, S.X. and B.Y.; Funding Acquisition, S.X. and B.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Fundamental Research Funds for Central Public Research Institutes of China, grant number 2022YSKY-14 and Fundamental Research Funds for the Central Public-interest Scientific Institution, grant number 2023YSKY-07.

Data Availability Statement: No new data were created or analyzed in this study. No AI tools were used to generate text or images.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Ahmad, M.; Rajapaksha, A.U.; Lim, J.E.; Zhang, M.; Bolan, N.; Mohan, D.; Vithanage, M.; Lee, S.S.; Ok, Y.S. Biochar as a sorbent for contaminant management in soil and water: A review. *Chemosphere* **2014**, *99*, 19–33. [[CrossRef](#)] [[PubMed](#)]
2. Meyer, S.; Glaser, B.; Quicker, P. Technical, economical, and climate-related aspects of biochar production technologies: A literature review. *Environ. Sci. Technol.* **2011**, *45*, 9473–9483. [[CrossRef](#)] [[PubMed](#)]
3. Glaser, B.; Haumaier, L.; Guggenberger, G.; Zech, W. The ‘Terra Preta’ phenomenon: A model for sustainable agriculture in the humid tropics. *Naturwissenschaften* **2001**, *88*, 37–41. [[CrossRef](#)] [[PubMed](#)]
4. Spokas, K.A.; Cantrell, K.B.; Novak, J.M.; Archer, D.W.; Ippolito, J.A.; Collins, H.P.; Boateng, A.A.; Lima, I.M.; Lamb, M.C.; McAloon, A.J. Biochar: A synthesis of its agronomic impact beyond carbon sequestration. *J. Environ. Qual.* **2012**, *41*, 973–989. [[CrossRef](#)] [[PubMed](#)]
5. Bridgwater, A. The production of biofuels and renewable chemicals by fast pyrolysis of biomass. *Int. J. Glob. Energy Issues* **2007**, *27*, 160–203. [[CrossRef](#)]
6. Ji, C.; Li, S.; Geng, Y.; Yuan, Y.; Zhi, J.; Yu, K.; Han, Z.; Wu, S.; Liu, S.; Zou, J. Decreased N₂O and NO emissions associated with stimulated denitrification following biochar amendment in subtropical tea plantations. *Geoderma* **2020**, *365*, 114223. [[CrossRef](#)]
7. Chen, C.; Su, J.; Ali, A.; Zhai, Z. Cornstalk biochar promoted the denitrification performance and cellulose degradation rate of *Burkholderia* sp. CF6. *J. Environ. Chem. Eng.* **2022**, *10*, 106998. [[CrossRef](#)]
8. Dai, Z.; Li, Y.; Zhang, X.; Wu, J.; Luo, Y.; Kuzyakov, Y.; Brookes, P.C.; Xu, J. Easily mineralizable carbon in manure-based biochar added to a soil influences N₂O emissions and microbial-N cycling genes. *Land Degrad. Dev.* **2018**, *30*, 406–416. [[CrossRef](#)]
9. Gao, D.; Tian, H.; Yang, H.; Tan, W.; Liang, X.; Wen, C.; Song, D.; Zhang, Y.; Tan, Q. Co-application of digestate and biochar reduced greenhouse gas emissions in paddy soil through enhanced denitrification and anaerobic methane oxidation. *J. Environ. Manag.* **2025**, *381*, 125244. [[CrossRef](#)] [[PubMed](#)]
10. Zhang, X.; Cui, X.; Dong, C.; Cai, M.; Wu, Q.; Jin, M. Carbon slow release and enhanced denitrification performance and microbial function of sludge-based biochar composite filler in SBR application. *J. Environ. Chem. Eng.* **2025**, *13*, 118507. [[CrossRef](#)]
11. Tong, H.; Duan, Z.; Yang, X.; Li, E.; Liu, Y.; Zhou, H.; Zhang, X.; Yang, X.; Xu, W. Characteristics of sludge-based pyrolysis biochar and its application of enhancing denitrification. *Chemosphere* **2022**, *309*, 136813. [[CrossRef](#)] [[PubMed](#)]
12. Zhang, Z.; Zhu, Z.; Shen, B.; Liu, L. Insights into biochar and hydrochar production and applications: A review. *Energy* **2019**, *171*, 581–598. [[CrossRef](#)]
13. Sathishkumar, K.; Li, Y.; Sanganyado, E. Electrochemical behavior of biochar and its effects on microbial nitrate reduction: Role of extracellular polymeric substances in extracellular electron transfer. *Chem. Eng. J.* **2020**, *395*, 125077. [[CrossRef](#)]
14. Raja, S.A.; Kennedy, Z.R.; Pillai, B.; Lee, C.L.R. Flash pyrolysis of jatropha oil cake in fluidized bed reactor. *Electron. Newsl. Renew. Energy Environ.* **2009**, *3*, 753.
15. Torr, K.M.; de Miguel Mercader, F.; Murton, K.D.; Harbers, T.J.; Cooke-Willis, M.H.; van de Pas, D.J.; Suckling, I.D. Fast pyrolysis of pine wood pretreated by large pilot-scale thermomechanical refining for biochemical production. *Ind. Eng. Chem. Res.* **2020**, *59*, 21294–21304. [[CrossRef](#)]
16. Cheng, C.H.; Lehmann, J.; Thies, J.E.; Burton, S.D. Stability of black carbon in soils across a climatic gradient. *J. Geophys. Res. Biogeosci.* **2008**, *113*. [[CrossRef](#)]
17. Cobbina, S.; Duwiejuah, A.; Quainoo, A. Single and simultaneous adsorption of heavy metals onto groundnut shell biochar produced under fast and slow pyrolysis. *Int. J. Environ. Sci. Technol.* **2019**, *16*, 3081–3090.

18. Liang, Y.; Wang, Q.; Huang, L.; Liu, M.; Wang, N.; Chen, Y. Insight into the mechanisms of biochar addition on pollutant removal enhancement and nitrous oxide emission reduction in subsurface flow constructed wetlands: Microbial community structure, functional genes and enzyme activity. *Bioresour. Technol.* **2020**, *307*, 123249. [[CrossRef](#)] [[PubMed](#)]
19. Chen, Q.; Tan, X.; Liu, Y.; Liu, S.; Li, M.; Gu, Y.; Zhang, P.; Ye, S.; Yang, Z.; Yang, Y. Biomass-derived porous graphitic carbon materials for energy and environmental applications. *J. Mater. Chem. A* **2020**, *8*, 5773–5811. [[CrossRef](#)]
20. Kumar, G.; Shobana, S.; Chen, W.-H.; Bach, Q.-V.; Kim, S.-H.; Atabani, A.; Chang, J.-S. A review of thermochemical conversion of microalgal biomass for biofuels: Chemistry and processes. *Green Chem.* **2017**, *19*, 44–67. [[CrossRef](#)]
21. Keiluweit, M.; Nico, P.S.; Johnson, M.G.; Kleber, M. Dynamic molecular structure of plant biomass-derived black carbon (biochar). *Environ. Sci. Technol.* **2010**, *44*, 1247–1253. [[CrossRef](#)] [[PubMed](#)]
22. Mayakaduwa, S.; Herath, I.; Ok, Y.S.; Mohan, D.; Vithanage, M. Insights into aqueous carbofuran removal by modified and non-modified rice husk biochars. *Environ. Sci. Pollut. Res.* **2017**, *24*, 22755–22763.
23. Yang, J.; Li, H.; Zhang, D.; Wu, M.; Pan, B. Limited role of biochars in nitrogen fixation through nitrate adsorption. *Sci. Total Environ.* **2017**, *592*, 758–765. [[CrossRef](#)] [[PubMed](#)]
24. Klüpfel, L.; Keiluweit, M.; Kleber, M.; Sander, M. Redox properties of plant biomass-derived black carbon (biochar). *Environ. Sci. Technol.* **2014**, *48*, 5601–5611. [[CrossRef](#)] [[PubMed](#)]
25. Chen, G.; Zhang, Z.; Zhang, Z.; Zhang, R. Redox-active reactions in denitrification provided by biochars pyrolyzed at different temperatures. *Sci. Total Environ.* **2018**, *615*, 1547–1556. [[CrossRef](#)] [[PubMed](#)]
26. Szczeńniak, B.; Phuriragpitikhon, J.; Choma, J.; Jaroniec, M. Recent advances in the development and applications of biomass-derived carbons with uniform porosity. *J. Mater. Chem. A* **2020**, *8*, 18464–18491. [[CrossRef](#)]
27. Leng, L.J.; Xiong, Q.; Yang, L.H.; Li, H.; Zhou, Y.Y.; Zhang, W.J.; Jiang, S.J.; Li, H.L.; Huang, H.J. An overview on engineering the surface area and porosity of biochar. *Sci. Total Environ.* **2021**, *763*, 144204. [[CrossRef](#)] [[PubMed](#)]
28. Aziz, K.H.H.; Mustafa, F.S.; Hassan, M.A.; Omer, K.M.; Hama, S. Biochar as green adsorbents for pharmaceutical pollution in aquatic environments: A review. *Desalination* **2024**, *583*, 117725. [[CrossRef](#)]
29. Hollister, C.C.; Bisogni, J.J.; Lehmann, J. Ammonium, nitrate, and phosphate sorption to and solute leaching from biochars prepared from corn stover (*Zea mays* L.) and oak wood (*Quercus* spp.). *J. Environ. Qual.* **2013**, *42*, 137–144. [[CrossRef](#)] [[PubMed](#)]
30. Joshi, M.; Bhatt, D.; Srivastava, A. Enhanced adsorption efficiency through biochar modification: A comprehensive review. *Ind. Eng. Chem. Res.* **2023**, *62*, 13748–13761. [[CrossRef](#)]
31. Švábová, M.; Bičáková, O.; Vorokhta, M. Biochar as an effective material for acetone sorption and the effect of surface area on the mechanism of sorption. *J. Environ. Manag.* **2023**, *348*, 119205. [[CrossRef](#)]
32. Tomczyk, A.; Kondracki, B.; Szewczuk-Karpisz, K. Chemical modification of biochars as a method to improve its surface properties and efficiency in removing xenobiotics from aqueous media. *Chemosphere* **2023**, *312*, 137238. [[CrossRef](#)] [[PubMed](#)]
33. Yang, K.; Peng, J.; Srinivasakannan, C.; Zhang, L.; Xia, H.; Duan, X. Preparation of high surface area activated carbon from coconut shells using microwave heating. *Bioresour. Technol.* **2010**, *101*, 6163–6169. [[CrossRef](#)] [[PubMed](#)]
34. Liu, Z.; Xue, Y.; Gao, F.; Cheng, X.; Yang, K. Removal of ammonium from aqueous solutions using alkali-modified biochars. *Chem. Speciat. Bioavailab.* **2016**, *28*, 26–32. [[CrossRef](#)]
35. Chintala, R.; Mollinedo, J.; Schumacher, T.E.; Papiernik, S.K.; Malo, D.D.; Clay, D.E.; Kumar, S.; Gulbrandson, D.W. Nitrate sorption and desorption in biochars from fast pyrolysis. *Microporous Mesoporous Mater.* **2013**, *179*, 250–257. [[CrossRef](#)]
36. Fan, Y.; Sun, S.; Gu, X.; Zhang, M.; Peng, Y.; Yan, P.; He, S. Boosting the denitrification efficiency of iron-based constructed wetlands in-situ via plant biomass-derived biochar: Intensified iron redox cycle and microbial responses. *Water Res.* **2024**, *253*, 121285. [[CrossRef](#)] [[PubMed](#)]
37. Jia, W.; Sun, X.; Gao, Y.; Yang, Y.; Yang, L. Fe-modified biochar enhances microbial nitrogen removal capability of constructed wetland. *Sci. Total Environ.* **2020**, *740*, 139534. [[CrossRef](#)] [[PubMed](#)]
38. Zhang, M.; Gao, B.; Yao, Y.; Xue, Y.; Inyang, M. Synthesis of porous MgO-biochar nanocomposites for removal of phosphate and nitrate from aqueous solutions. *Chem. Eng. J.* **2012**, *210*, 26–32. [[CrossRef](#)]
39. Dewage, N.B.; Liyanage, A.S.; Pittman, C.U., Jr.; Mohan, D.; Mlsna, T. Fast nitrate and fluoride adsorption and magnetic separation from water on α -Fe₂O₃ and Fe₃O₄ dispersed on Douglas fir biochar. *Bioresour. Technol.* **2018**, *263*, 258–265. [[CrossRef](#)]
40. Chen, J.; Zhou, J.; Zheng, W.; Leng, S.; Ai, Z.; Zhang, W.; Yang, Z.; Yang, J.; Xu, Z.; Cao, J. A complete review on the oxygen-containing functional groups of biochar: Formation mechanisms, detection methods, engineering, and applications. *Sci. Total Environ.* **2024**, *946*, 174081. [[CrossRef](#)] [[PubMed](#)]
41. Xu, J.; Wu, X.; Zhu, N.; Shen, Y.; Yuan, H. Anammox process dosed with biochars for enhanced nitrogen removal: Role of surface functional groups. *Sci. Total Environ.* **2020**, *748*, 141367. [[CrossRef](#)] [[PubMed](#)]
42. Lin, Y.; Li, Y.; Xu, Z.; Xiong, J.; Zhu, T. Transformation of functional groups in the reduction of NO with NH₃ over nitrogen-enriched activated carbons. *Fuel* **2018**, *223*, 312–323. [[CrossRef](#)]
43. Jia, W.; Yang, L. Community composition and spatial distribution of N-removing microorganisms optimized by Fe-modified biochar in a constructed wetland. *Int. J. Environ. Res. Public Health* **2021**, *18*, 2938. [[CrossRef](#)] [[PubMed](#)]

44. Xiong, J.; Ren, S.; He, Y.; Wang, X.C.; Bai, X.; Wang, J.; Dzakpasu, M. Bioretention cell incorporating Fe-biochar and saturated zones for enhanced stormwater runoff treatment. *Chemosphere* **2019**, *237*, 124424. [[CrossRef](#)] [[PubMed](#)]
45. Feng, X.; Liu, S.; Yue, K.; Wei, H.; Bi, D.; Zhao, W. Insight into the promotional effect of Mn-modified nitrogenous biochar on the NH₃-SCR denitrification activity at low temperatures. *Energy* **2023**, *285*, 129323. [[CrossRef](#)]
46. Xu, L.; Su, J.; Ali, A.; Huang, T.; Yang, Y.; Shi, J.; Liang, E. Magnetite-loaded rice husk biochar promoted the denitrification performance of *Aquabacterium* sp. XL4 under low carbon to nitrogen ratio: Optimization and mechanism. *Bioresour. Technol.* **2022**, *348*, 126802. [[CrossRef](#)] [[PubMed](#)]
47. Fu, C.; Li, J.; Lv, X.; Song, W.; Zhang, X. Operation performance and microbial community of sulfur-based autotrophic denitrification sludge with different sulfur sources. *Environ. Geochem. Health* **2020**, *42*, 1009–1020. [[CrossRef](#)] [[PubMed](#)]
48. Fernández-Nava, Y.; Marañon, E.; Soons, J.; Castrillón, L. Denitrification of wastewater containing high nitrate and calcium concentrations. *Bioresour. Technol.* **2008**, *99*, 7976–7981. [[CrossRef](#)] [[PubMed](#)]
49. Su, X.; Chen, Y.; Wang, Y.; Yang, X.; He, Q. Impacts of chlorothalonil on denitrification and N₂O emission in riparian sediments: Microbial metabolism mechanism. *Water Res.* **2019**, *148*, 188–197. [[CrossRef](#)] [[PubMed](#)]
50. Lu, H.; Chandran, K.; Stensel, D. Microbial ecology of denitrification in biological wastewater treatment. *Water Res.* **2014**, *64*, 237–254. [[CrossRef](#)] [[PubMed](#)]
51. Zhang, W.; Shen, J.; Zhang, H.; Zheng, C.; Wei, R.; Gao, Y.; Yang, L. Efficient nitrate removal by *Pseudomonas mendocina* GL6 immobilized on biochar. *Bioresour. Technol.* **2021**, *320*, 124324. [[CrossRef](#)] [[PubMed](#)]
52. Harter, J.; Krause, H.-M.; Schuetzler, S.; Ruser, R.; Fromme, M.; Scholten, T.; Kappler, A.; Behrens, S. Linking N₂O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. *ISME J.* **2014**, *8*, 660–674. [[PubMed](#)]
53. Cayuela, M.L.; Sánchez-Monedero, M.A.; Roig, A.; Hanley, K.; Enders, A.; Lehmann, J. Biochar and denitrification in soils: When, how much and why does biochar reduce N₂O emissions? *Sci. Rep.* **2013**, *3*, 1732. [[CrossRef](#)] [[PubMed](#)]
54. Wu, Z.; Xu, F.; Yang, C.; Su, X.; Guo, F.; Xu, Q.; Peng, G.; He, Q.; Chen, Y. Highly efficient nitrate removal in a heterotrophic denitrification system amended with redox-active biochar: A molecular and electrochemical mechanism. *Bioresour. Technol.* **2019**, *275*, 297–306. [[CrossRef](#)] [[PubMed](#)]
55. Yuan, Y.; Bolan, N.; Prévosteau, A.; Vithanage, M.; Biswas, J.K.; Ok, Y.S.; Wang, H. Applications of biochar in redox-mediated reactions. *Bioresour. Technol.* **2017**, *246*, 271–281. [[CrossRef](#)] [[PubMed](#)]
56. Yu, L.; Wang, Y.; Yuan, Y.; Tang, J.; Zhou, S. Biochar as electron acceptor for microbial extracellular respiration. *Geomicrobiol. J.* **2016**, *33*, 530–536. [[CrossRef](#)]
57. Zhang, Y.; Zhang, Z.; Chen, Y. Biochar mitigates N₂O emission of microbial denitrification through modulating carbon metabolism and allocation of reducing power. *Environ. Sci. Technol.* **2021**, *55*, 8068–8078. [[CrossRef](#)] [[PubMed](#)]
58. da Silva, M.E.R.; Firmino, P.I.M.; dos Santos, A.B. Impact of the redox mediator sodium anthraquinone-2, 6-disulphonate (AQDS) on the reductive decolourisation of the azo dye Reactive Red 2 (RR2) in one-and two-stage anaerobic systems. *Bioresour. Technol.* **2012**, *121*, 1–7. [[CrossRef](#)] [[PubMed](#)]
59. Cayuela, M.L.; Spott, O.; Pascual, M.B.; Sánchez-García, M.; Sánchez-Monedero, M.A. Key biochar properties linked to denitrification products in a calcareous soil. *Biochar* **2024**, *6*, 90. [[CrossRef](#)]
60. Yuan, D.; Wu, P.; Yuan, J.; Jia, Z.; Hu, C.; Clough, T.J.; Wrage-Mönnig, N.; Luo, J.; Tang, J.; Qin, S. Loading of redox-active metal Fe largely enhances the capacity of biochar to mitigate soil N₂O emissions by promoting complete denitrification. *Biol. Fertil. Soils* **2024**, *61*, 681–693. [[CrossRef](#)]
61. Li, R.M.; Li, Z.Y.; Zhou, J.M.; Zhang, Y.N.; Whang, Z.; Zou, Y.; Jin, R.C.; Zhang, Q.Q. Insights into the effect of biochar on single-stage partial denitrification/anaerobic ammonium oxidation process: Nitrogen removal performance and mechanism. *Bioresour. Technol.* **2026**, *444*, 133963. [[CrossRef](#)] [[PubMed](#)]
62. Yu, Y.; An, Q.; Zhou, Y.; Deng, S.; Miao, Y.; Zhao, B.; Yang, L. Highly synergistic effects on ammonium removal by the co-system of *Pseudomonas stutzeri* XL-2 and modified walnut shell biochar. *Bioresour. Technol.* **2019**, *280*, 239–246. [[CrossRef](#)] [[PubMed](#)]
63. Chen, W.; Zhang, L.; Liu, Z.; Liu, W.; Lu, B.; Zhao, H. Mechanism of nano-scale zero-valent iron modified biochar for enhancing low-nitrogen anammox process resistance to low temperatures. *J. Environ. Sci.* **2025**, *152*, 442–452. [[CrossRef](#)]
64. Ming, J.; Wang, Q.; Yoza, B.A.; Liang, J.; Guo, H.; Li, J.; Guo, S.; Chen, C. Bioreactor performance using biochar and its effect on aerobic granulation. *Bioresour. Technol.* **2020**, *300*, 122620. [[CrossRef](#)] [[PubMed](#)]
65. Wang, X.; Li, J.; Liu, R.; Hai, R.; Zou, D.; Zhu, X.; Luo, N. Responses of bacterial communities to CuO nanoparticles in activated sludge system. *Environ. Sci. Technol.* **2017**, *51*, 5368–5376. [[CrossRef](#)] [[PubMed](#)]
66. Deng, C.; Huang, L.; Liang, Y.; Xiang, H.; Jiang, J.; Wang, Q.; Hou, J.; Chen, Y. Response of microbes to biochar strengthen nitrogen removal in subsurface flow constructed wetlands: Microbial community structure and metabolite characteristics. *Sci. Total Environ.* **2019**, *694*, 133687. [[CrossRef](#)] [[PubMed](#)]
67. Liu, Y.; Gan, L.; Chen, Z.; Megharaj, M.; Naidu, R. Removal of nitrate using *Paracoccus* sp. YF1 immobilized on bamboo carbon. *J. Hazard. Mater.* **2012**, *229*, 419–425. [[CrossRef](#)] [[PubMed](#)]

68. Zhao, W.; Cheng, C.; Xiao, H.; Zheng, H.; Zhou, T.; Ding, Y.; Chai, H. Mechanism exploration of biochar-mitigated nitrous oxide emission in pyrite-driven autotrophic denitrification system: Generation-transfer-consumption of electrons. *Bioresour. Technol.* **2026**, *445*, 134072. [[CrossRef](#)] [[PubMed](#)]
69. Berger, A.W.; Valenca, R.; Miao, Y.; Ravi, S.; Mahendra, S.; Mohanty, S.K. Biochar increases nitrate removal capacity of woodchip biofilters during high-intensity rainfall. *Water Res.* **2019**, *165*, 115008. [[CrossRef](#)] [[PubMed](#)]
70. Jia, Y.; Zhang, H.; Gu, T.; Wu, Y.; Yang, Z.; Shao, Y.; Zhong, H. Denitrification in bioretention systems based on corncob biochar produced at low pyrolysis temperature: The efficacy and the mechanisms. *Chem. Eng. J.* **2023**, *460*, 141829. [[CrossRef](#)]
71. Jiang, G.; Liu, Y.; Liu, X.; Shen, Y.; Zhang, A.; Li, Z. Enhanced efficiency and mechanism of low-temperature biochar on simultaneous removal of nitrogen and phosphorus by combined heterotrophic nitrification-aerobic denitrification bacteria. *Bioresour. Technol.* **2023**, *373*, 128720. [[CrossRef](#)] [[PubMed](#)]
72. Wang, Z.; Gu, Z.; Yang, Y.; Chen, Y.; Dai, B.; Wang, X.; Xia, S. Inhibition, recovery and stabilization of MBR-anammox process: Enhancing nitrogen removal by biochar-supported nano zero-valent iron adoption. *Chem. Eng. J.* **2024**, *495*, 153476. [[CrossRef](#)]
73. Li, P.; Lin, K.; Fang, Z.; Wang, K. Enhanced nitrate removal by novel bimetallic Fe/Ni nanoparticles supported on biochar. *J. Clean. Prod.* **2017**, *151*, 21–33. [[CrossRef](#)]
74. Chen, Y.; Yu, B.; Lin, J.; Naidu, R.; Chen, Z. Simultaneous adsorption and biodegradation (SAB) of diesel oil using immobilized *Acinetobacter venetianus* on porous material. *Chem. Eng. J.* **2016**, *289*, 463–470. [[CrossRef](#)]
75. Mao, Q.; Bao, J.; Du, J.; He, T.; Zhang, Y.; Cheng, B. Biochar enhanced the stability and microbial metabolic activity of aerobic denitrification system under long-term oxytetracycline stress. *Bioresour. Technol.* **2023**, *382*, 129188. [[CrossRef](#)] [[PubMed](#)]
76. Liu, Y.; Liu, S.; Yang, Z.; Xiao, L. Synergetic effects of biochars and denitrifier on nitrate removal. *Bioresour. Technol.* **2021**, *335*, 125245. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.