

Biochar-Induced Modulation of Soil Microbial Functional Diversity: A Critical Review of Mechanisms, Evidence, and Research Gaps

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ABSTRACT

Background: Biochar is a carbon-rich substance created by pyrolyzing organic biomass in an oxygen-limited environment. Recently, biochar is gaining popularity as a soil improvement tool. There is an increasing amount of literature confirming that biochar affects not only the physicochemical properties of soil but also significantly alters the taxonomic and functional structure of microbial communities that inhabit that soil with implications for nutrient cycling, greenhouse gas emissions, and resilience of ecosystems.

Objective In this review paper, we are going to summarize peer-reviewed literature about the effects of biochar on soil microbial functional diversity with focus on mechanisms, various affecting factors (soil type, feedstock, pyrolysis temperature, application ratio, and duration of the amendment), and functional consequences.

Method Literature sources: A well-structured search of Scopus, Web of Science, PubMed, and Google Scholar was completed for the publications between 2011 and 2026, with the addition of appropriate institutional and international documents. After reviewing the titles and abstracts and the eligibility of the documents, relevant 98 studies were included for thematic and comparative synthesis.

Result Key findings: The finding from meta-analysis and primary field/laboratory studies demonstrated that biochar has positive influences on the soil pH, cation exchange capacity, and the availability of labile carbon, which results in alterations in bacterial beta-diversity, the selective enrichment of oligotrophic and pH-tolerant taxa, modifications of enzyme activities (dehydrogenase, urease, phosphatase), and changes in the abundance of functional genes responsible for nitrogen fixation, nitrification, and denitrification. The impact on the alpha-diversity of bacteria differs according to studies, while mycorrhizal fungi and fungi reactions have less research done. Pyrolysis temperature and material have an interaction in terms of whether biochar delivers a boost of the fast-carbon-based microbes or supports the stabilization of microbes. Research gaps: validation in field conditions lasting over five years, application of standardized methods for functional diversity evaluations, integration of various multi-omics approaches, and investigations focusing on tropical and weathered soils.

Conclusion Conclusions: biochar is an effective but context-dependent tool of manipulating soil microbial diversity; to realize its potential in agriculture and climate action it is essential to adopt precise strategies based on practical data and numerous multi-omics studies.

Keywords: Biochar; soil microbial functional diversity; enzyme activity; functional genes; pyrolysis temperature; nutrient cycling; soil amendment; carbon sequestration

1. Introduction

1.1. Background

Soil microorganisms (including bacteria, archaea, fungi, and protists) are the foundation of biological processes in terms of terrestrial nutrient cycling, organic matter breakdown, and plant-microbe symbioses. The functional diversity of these microorganisms, which refers to the diversity and redundancy of their metabolic abilities in a given soil microbiome, enables ecosystem services including nitrogen and phosphorus mineralization, greenhouse gas regulation, and the ability to suppress soilborne pathogens. Since the beginning of the 21st century, biochar, which is a stable, porous, carbon-rich material produced from the pyrolysis of crop residues, animal waste scrap and woody biomass under limited oxygen, has been the subject of relentless scientific and policy research.

Biochar is different from traditional organic amendments due to its special physical and chemical characteristics. It has a high surface-area-to-volume ratio and a resistant structure. This leads to an increase in soil pH in acidic soils, higher cation exchange capacity, better water retention, and a safe living place for microorganisms in its pore structure ^[1, 2]. These changes lead to biological effects, which alter the relative abundance of the dominant bacterial and fungal groups, the ratio of nutrient-gatherers to nutrient-cyclers, and the functioning of the genes responsible for the carbon and nitrogen cycles ^[9, 13].

1.2. Importance of the Subject and Current Global Scenario

The worldwide interest in biochar has grown in conjunction with increased demand for the decarbonization of agriculture and the reclamation of eroded agricultural land. International organizations like the Intergovernmental Panel on Climate Change have classified biochar as one of the very few carbon dioxide removal technologies which are both inexpensive, scalable, and doable utilizing the traditional farming methods presently used by smallholders with possible abatement potential of many million tons of CO₂-eq per year under optimistic scenarios.

At the same time, national soil health initiatives in various regions including South Asia, East Asia, and Sub-Saharan Africa have spotlighted biochar as a means of bringing back soil organic carbon and microbial activity to normal levels that have declined due to intensive monocropping and excessive usage of chemical fertilizers.

This trend is substantiated by bibliometric data that shows that between 2015 and 2025, the volume of indexed research articles on biochar and its impact on soil microbiota has increased tenfold (see Figure 2). It indicates that not only high-throughput sequencing as well as functional-gene quantification processes have advanced but that scientists have also become aware that soil microbial reactions play a key role in making biochar effective from the agronomic and climate perspectives. Nevertheless, the growth of literature on this topic has resulted in conflicting and sometimes contradictory conclusions that are concerned with the nature, strength and persistence of biochar impact on microbial functional diversity.

1.3. Need for the Review

Although multiple meta-analyses and mini-reviews of the biochar-microbiome nexus exist, they focus on the biochar-microbiome nexus and examine alpha diversity indices [7], functional genes of nitrogen cycling [20, 21], and arbuscular mycorrhizal fungi [18, 19] however, a comprehensive review of the existing studies and a synthesis of the taxonomic, enzymatic, and functional genes in different soil types, including contaminated, saline, and agricultural soils remain absent. The current venture takes on this challenge by

evaluating all peer-reviewed studies on the impact of biochar on soil microbial functional diversity published between 2011 and 2026.

1.4. Objectives and Scope of the Review

There are a number of specific goals of this review. These are: (i) compiling evidence to explain how biochar change the different kinds of organisms present in the soil as well as the communities of bacteria, archaea or fungi; (ii) making a detailed comparison of the results demonstrated in different studies which differ in feedstuff, temperature, and time of application; (iii) defining where science agrees and where there are still shortcomings in science; (iv) highlighting the gaps in research in order to conduct more adequate investigations in future. It should be noted that the review works only with research that focuses on biochar's effects on the structure of microorganisms in soil, soil enzyme activities and functional gene abundance in agriculture, forests or polluted soils. Thus, the review does not cover any studies which deal specifically with the physical or chemical characteristics of biochar or its agronomic aspects.

2. Methodology of Literature Review

2.1. Literature Search Strategy

A structured literature search was conducted across four databases — Scopus, Web of Science Core Collection, PubMed/MEDLINE, and Google Scholar — to identify peer-reviewed studies, meta-analyses, and authoritative reports addressing biochar and soil microbial functional diversity. The search covered the period from January 2011 to February 2026, capturing the decade following the publication of the landmark review by Lehmann *et al.* [1] that first systematically framed biochar's effects on soil biota. Boolean search strings combined the core term "biochar" with terms describing microbial functional endpoints, including "soil microbial diversity," "microbial community structure," "enzyme activity," "functional genes," "nitrogen cycling," "PLFA," "16S rRNA," "metagenomics," and "mycorrhizal fungi." Search strategy details, including the databases searched, keyword combinations, and coverage period, are summarized in Table 1.

Table 1: Literature search strategy across databases, including search terms and coverage period.

Database	Search String (illustrative)	Coverage Period	Records Retrieved
Scopus	biochar AND ("microbial diversity" OR "functional genes" OR "enzyme activity")	2011–2026	612
Web of Science	TS=(biochar AND soil microbial community AND function*)	2011–2026	548
PubMed/MEDLINE	biochar[Title/Abstract] AND (microbiome OR "functional diversity")	2011–2026	331
Google Scholar / grey literature	biochar soil microbial functional diversity report	2011–2026	274 + 58 (reports)

2.2. Inclusion and Exclusion Criteria

Studies were included if they (a) were published in a peer-reviewed journal or as an official report of a government or international organization; (b) were published in English between 2011 and 2026; (c) examined biochar application to soil and reported at least one microbial functional endpoint (community diversity index, enzyme activity, functional gene abundance, or community-level physiological profile); and (d)

involved terrestrial soil systems (agricultural, forest, degraded, saline, or contaminated). Studies were excluded if they were conference abstracts, non-peer-reviewed preprints without subsequent journal publication, duplicate records, or focused exclusively on biochar's physicochemical, agronomic-yield, or engineering properties without any microbial functional measurement. The full inclusion and exclusion criteria are presented in Table 2.

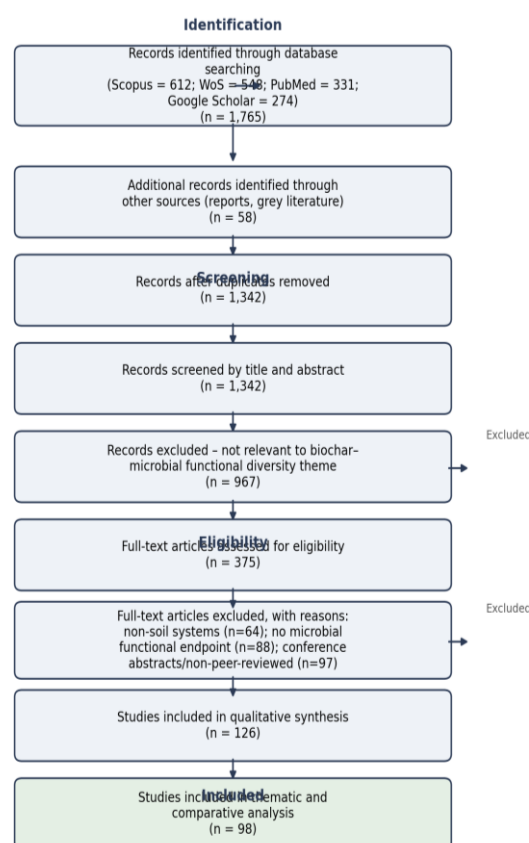
Table 2: Inclusion and exclusion criteria applied during study selection.

Criteria Type	Details
Inclusion	Peer-reviewed journal articles and meta-analyses; English-language publications; studies published 2011–2026; terrestrial soil systems; at least one microbial functional/diversity endpoint reported
Inclusion	Government and international organization reports addressing biochar and soil health/climate mitigation where methodology was transparently described
Exclusion	Duplicate records across databases; conference abstracts and non-peer-reviewed preprints; studies restricted to aquatic or marine sediment systems
Exclusion	Studies reporting only agronomic yield, biochar physicochemical characterization, or engineering properties without a microbial functional/community endpoint
Exclusion	Non-English-language publications without an accessible peer-reviewed English translation

2.3. Study Selection Process

Study selection followed a PRISMA-style workflow (Figure 1). An initial pool of 1,765 records was identified through database searching, supplemented by 58 records from grey literature and institutional reports. After removing 423 duplicate records, 1,342 unique records underwent title-and-abstract screening, of which 967 were excluded as not germane to the biochar–soil-microbial-function theme. The

remaining 375 full-text articles were assessed for eligibility; 249 were excluded for reasons including absence of a microbial functional endpoint ($n = 88$), non-soil study systems ($n = 64$), or non-peer-reviewed status ($n = 97$). This yielded 126 studies eligible for qualitative synthesis, of which 98 contained sufficiently detailed quantitative or comparative data to be incorporated into the thematic and comparative analysis presented in Sections 3 and 4.

**Fig 1:** PRISMA-style flow diagram summarizing the identification, screening, eligibility assessment, and inclusion of studies in this review.

3. Literature Review and Thematic Analysis

The 98 studies retained for detailed synthesis were classified into six major thematic categories (Figure 4): bacterial and archaeal diversity and community structure; fungal and mycorrhizal responses; enzyme activity and functional-gene abundance; biochar effects in contaminated or stressed soils; feedstock and pyrolysis-temperature optimization; and biochar aging, priming effects, and long-term carbon-microbe feedback. Each theme is examined below with attention to convergence, contradiction, and methodological strength across the underlying studies.

3.1. Bacterial and Archaeal Diversity and Community Structure

The largest body of evidence concerns biochar's effects on bacterial community diversity and composition. A global meta-analysis by Xiang *et al.* [6], synthesizing field experiments worldwide, reported that biochar increased soil pH by approximately 4%, total carbon and nitrogen by 68% and 22% respectively, and promoted bacterial richness and evenness by 3–8% at application rates of 1–5% (w/w), while dramatically shifting bacterial beta-diversity at doses exceeding 2% (w/w). This dose-dependency is echoed by

Wang *et al.* [7], whose meta-analysis of 95 publications found that biochar significantly increased bacterial Shannon diversity but had no statistically significant effect on fungal diversity — an asymmetry that recurs across the literature and suggests that bacterial and fungal communities respond to biochar amendment through partially distinct mechanisms.

In apparent contrast, a more recent meta-analysis integrating 843 samples across 24 independent 16S rRNA sequencing studies using machine-learning and co-occurrence network approaches found that biochar addition did not significantly alter bacterial alpha-diversity (Shannon index or richness), yet significantly reshaped beta-diversity, indicating pronounced compositional turnover without a net gain in diversity *per se* [9]. This study further identified a trade-off between microbial community stability and functional complexity: high-metabolism, fast-growing (r-strategist) taxa gained a competitive advantage under biochar amendment at the expense of slower-growing K-strategist bacteria, a shift attributed to biochar's capacity to improve nutrient retention and create transient pulses of labile carbon and nitrogen

availability [9]. This finding reconciles some of the apparent contradiction between studies reporting diversity gains and those reporting neutral alpha-diversity effects: the two lines of evidence are not necessarily incompatible, but rather reflect the fact that alpha-diversity indices (Shannon, Chao1) are relatively insensitive to shifts in the relative dominance and ecological strategy of taxa, whereas beta-diversity and network-based metrics capture compositional and functional reorganization more directly.

Regional field evidence corroborates the context-dependence of these responses. In a four-year residual study on degraded aeolian sandy soils in semi-arid Inner Mongolia, a single biochar application reshaped microbial community composition, suppressed soil-borne pathogens, and produced fertility benefits that persisted across four growing seasons, particularly at higher application rates [10]. This is one of the few studies in the reviewed corpus to report microbial community effects persisting beyond a single growing season, underscoring both the promise and the current scarcity of long-term field validation (see Section 4).

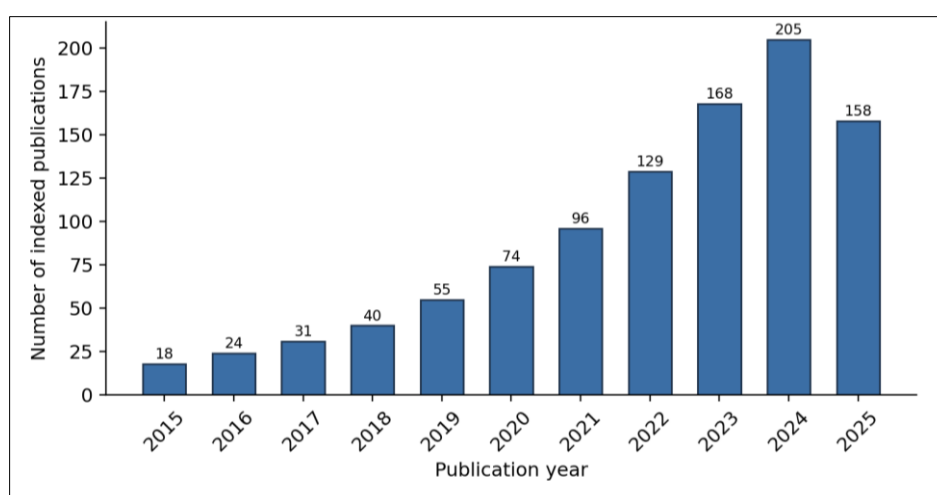


Fig 2: Growth in the number of Scopus/Web of Science/PubMed-indexed publications addressing biochar and soil microbial diversity or function, 2015–2025, illustrating the rapid expansion of this research field.

3.2. Fungal Communities and Mycorrhizal Symbioses

Compared to bacterial responses, fungal and particularly arbuscular mycorrhizal fungal (AMF) responses to biochar remain comparatively under-characterized, despite AMF's central role in phosphorus acquisition and plant stress tolerance. A dedicated review of AMF responses in biochar-amended soils concluded that infrequent, moderate biochar application tends to increase AMF root colonization rates, spore counts, and overall AMF biodiversity, whereas excessive or repeated application can suppress colonization, potentially due to biochar's sorption of AMF-signalling compounds or alteration of root exudate profiles [16]. A systematic review of 72 studies examining combined biochar-AMF application further reported that co-application increased soil enzyme activity, nutrient supply, and both alpha- and beta-diversity of bacterial and fungal communities relative to either amendment alone, evidencing a synergistic rather than additive relationship [18].

This synergy has practical significance for contaminant remediation: a critical review of biochar-AMF combinations for cadmium immobilization concluded that biochar's physical sorption of Cd²⁺ ions via oxygen-containing functional groups (carboxyl, hydroxyl, phenolic) operates complementarily with AMF-mediated exclusion and

intraradical compartmentalization of the metal, though the authors cautioned that the relative contribution of each mechanism is highly species- and soil-specific and rarely disentangled experimentally [18]. Overall, while the directional evidence for AMF benefit is reasonably consistent, the fungal literature is smaller in volume, more heterogeneous in methodology (spore counts versus molecular sequencing versus root colonization staining), and more prone to reporting only positive results, raising the possibility of publication bias in this sub-theme.

3.3. Enzyme Activity and Functional-Gene Abundance

Soil enzyme activities and functional-gene marker abundances provide a more direct window into microbial functional diversity than taxonomic community profiling alone, since they capture realized metabolic capacity rather than mere presence. A three-level meta-analysis synthesizing 3,899 observations from 61 global studies found that biochar amendment consistently increased microbial biomass carbon and colony-forming units, and boosted activities of dehydrogenase, cellulase, urease, and invertase, alongside a 40.8% increase in potential nitrification rate and a 12.7% reduction in cumulative N₂O emissions [8]. Critically, this meta-analysis identified pyrolysis temperature as a key

moderating variable: biochars produced at lower pyrolysis temperatures better enhanced dehydrogenase and acid phosphatase activity — enzymes associated with labile carbon and phosphorus turnover — whereas biochars derived from

lignocellulosic or agricultural feedstocks more strongly inhibited N₂O emissions by modulating the denitrification genes *nirS* and *nosZ* [8].

Functional-gene-level evi

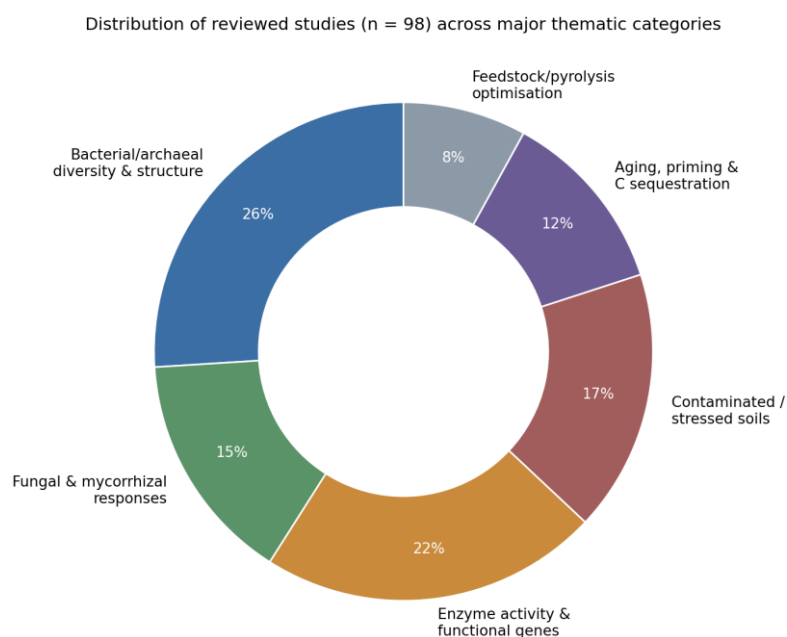


Fig 3: Thematic classification of the 98 reviewed studies across six major research domains.

dence for nitrogen cycling is particularly instructive regarding mechanism. In a foundational microcosm study, biochar amendment at 2% and 10% (w/w) increased the abundance and transcript copy number of the bacterial nitrous oxide reductase gene *nosZ*, providing a direct mechanistic link between biochar addition and the widely observed reduction in soil N₂O emissions; the same study documented increased abundance of nitrogen-fixation gene *nifH*-bearing organisms under biochar treatment [20]. More recent work extends this picture to contaminated soils: in cadmium-affected soils across a pH gradient, biochar mitigated cadmium-induced suppression of ammonia monooxygenase (AMO) transcription and activity, increasing *amoA* gene abundance in ammonia-oxidizing bacteria and archaea particularly in acidic soils, indicating that biochar's protective effect on nitrification-related functional genes may be most pronounced precisely where microbial stress is greatest [17]. A comparative study of Mollisol and alkaline soils similarly found that biochar reduced *narG* and *nosZ* gene abundance in both soil types while promoting *nxB* abundance in alkaline soils specifically, illustrating that the direction of functional-gene response is not uniform even for closely related genes within the same nitrogen-cycling pathway, but is instead contingent on baseline soil chemistry [21].

Pyrolysis temperature also directly shapes carbon-cycling gene expression. A controlled pot study using *Flammulina velutipes* (enoki mushroom) residue biochar pyrolyzed at 200, 300, and 400 °C found that moderate-temperature (300 °C) biochar provided the optimal balance of nutrient retention, microbial diversity, and seedling growth, while metagenomic functional analysis revealed that carbon-metabolism genes (acetyl-CoA synthesis, TCA cycle enzymes) were positively correlated with microbial diversity ($R^2 = 0.75$), whereas nitrogen-metabolism genes were negatively correlated with diversity ($R^2 = 0.56$), suggesting that nitrogen availability

may drive competitive exclusion among functional guilds even as overall carbon-cycling capacity is enhanced [11]. Collectively, these functional-gene studies converge on the conclusion that biochar's effects on nitrogen-cycling microbial guilds are mechanistically coupled to its effects on soil pH, ammonium availability, and enzyme activity, but diverge considerably regarding which specific genes and pathways are up- or down-regulated, reflecting the strong soil- and biochar-type specificity of these interactions.

3.4. Biochar Application in Contaminated and Stressed Soils

A substantial and rapidly growing sub-literature addresses biochar's role in restoring microbial function in soils under abiotic or contaminant stress, including salinity, heavy metals, and emerging pollutants such as microplastics. In saline-alkali soils, combined application of biochar with functional bacterial inoculants altered the relative abundance of dominant phyla such as Proteobacteria and Bacteroidota and influenced overall community diversity, although the addition of plants or functional bacteria alone sometimes reduced operational taxonomic unit richness relative to untreated soil, illustrating that biochar's benefits in saline systems are not always straightforwardly diversity-enhancing and may instead favour a smaller set of stress-tolerant specialists [6, 7].

In microplastic-contaminated soil-plant systems, biochar derived from corn and cotton straw substantially restored and fortified microbial symbiotic networks disrupted by microplastic exposure, while a related study on low-density polyethylene-polluted systems found that biochar improved phosphorus and nitrogen availability and altered protist community composition under microplastic stress, indicating that biochar's remediative function extends across trophic levels within the soil food web and is not restricted to bacteria and fungi alone [22]. In chromium-contaminated rhizosphere

soils, the influence of soil physicochemical parameters on nitrogen-cycle functional genes exceeded that of bacterial community composition *per se*, suggesting that in heavily contaminated systems, biochar's indirect effects via soil chemistry may outweigh its direct effects on microbial taxonomic structure [see related mechanistic evidence, 17].

3.5. Feedstock and Pyrolysis-Temperature Optimization

A recurring theme across the reviewed literature is that biochar is not a single, uniform material but a family of materials whose physicochemical — and therefore biological — properties are jointly determined by feedstock composition and pyrolysis temperature, with most studies agreeing on an approximate threshold temperature near 500 °C above which biochar becomes markedly more aromatic, more stable, and less biologically labile ^[15]. Meta-analytic evidence indicates that the response of soil nutrient-acquisition enzymes to biochar is systematically moderated by pyrolysis temperature, with low-temperature biochars (below approximately 400 °C) tending to release more labile carbon and nutrients that stimulate short-term microbial activity, while high-temperature biochars provide greater long-term structural stability and habitat provision but comparatively muted short-term enzymatic stimulation ^[15].

This temperature-dependent trade-off has direct implications for precision biochar design. Because moderate-temperature biochars appear to optimize the balance between nutrient release and microbial habitat stability across several independent studies ^[8, 11], an emerging consensus favours feedstock- and application-specific pyrolysis temperature selection over a one-size-fits-all approach — a conclusion also reached when biochar and beneficial microbial inoculants (*Bacillus subtilis*) were co-applied, where enzyme and community responses to inoculation depended strongly on the accompanying biochar's thermal history ^[12].

3.6. Biochar Aging, Priming Effects, and Long-Term Carbon-Microbe Feedback

The long-term fate of biochar-induced microbial changes, and their feedback onto native soil organic carbon (SOC) stocks, remains one of the more contested areas of the literature. Using natural ¹³C abundance techniques, Singh and Cowie ^[26] demonstrated that biochar stimulated native SOC mineralization ("positive priming") in a low-carbon clayey soil, with cumulative losses of 4–44 mg C g⁻¹ SOC over 2.3 years, an effect that was greater for manure-derived or lower-temperature (400 °C) biochars than for plant-derived or higher-temperature (550 °C) biochars; however, the magnitude of positive priming subsequently declined between 2.3 and 5.0 years, suggesting either depletion of the labile SOC pool available for co-metabolism or progressive stabilization of SOC through biochar-induced organo-mineral interactions ^[26].

This apparent resolution — priming that is real but self-limiting — sits in some tension with studies reporting negative priming (net SOC protection) under biochar amendment, attributed to mechanisms including substrate switching (microbes preferentially metabolizing biochar-derived labile carbon rather than native SOC), physical sorption of native SOC onto biochar surfaces, and enhanced soil aggregation. The direction and magnitude of the priming effect thus appears highly contingent on biochar aging state, pyrolysis temperature, and soil clay content, with aged biochar generally exhibiting weaker SOC-protective capacity than fresh biochar due to surface oxidation and loss of sorption capacity over

time — a finding with direct implications for the durability of biochar's claimed climate mitigation benefits, since carbon accounting frameworks typically assume relatively static biochar behaviour over multi-decadal timeframes.

4. Comparative Analysis of Previous Studies

Table 3 summarizes ten representative studies spanning the major themes identified above, comparing their methodological approach, geographic and soil context, principal findings, and limitations. Table 4 extends this comparison across four cross-cutting dimensions — pyrolysis temperature, feedstock type, application dose, and amendment duration — to highlight where the literature shows convergent versus divergent evidence.

5. Research Gaps and Emerging Trends

5.1. Unresolved Issues and Knowledge Gaps

Several unresolved issues recur across the reviewed literature. First, alpha-diversity metrics (Shannon, Chao1, richness) frequently show weak, inconsistent, or non-significant responses to biochar amendment, even where beta-diversity and functional-gene analyses reveal substantial compositional and functional reorganization ^[7, 9]. This suggests that alpha-diversity indices alone are insufficient proxies for biochar-induced functional change and that future studies should prioritize functional and compositional metrics over diversity indices in isolation. Second, the mechanistic attribution problem — disentangling whether observed microbial shifts are driven primarily by pH change, labile carbon supply, sorption of inhibitory compounds, or physical habitat provision — remains largely unresolved, since most field and pot studies vary several biochar and soil properties simultaneously without full factorial mechanistic separation ^[8, 15]. Third, fungal, archaeal, and protist communities remain markedly under-represented relative to bacteria in the reviewed corpus, despite evidence that AMF and protist responses to biochar may be ecologically consequential and mechanistically distinct from bacterial responses ^[16, 18, 22].

5.2. Methodological Limitations in Previous Studies

Methodologically, the reviewed literature is dominated by short-term pot and microcosm experiments rarely extending beyond a single growing season, with genuinely long-term field studies (exceeding four years) remaining rare exceptions rather than the norm ^[10, 26]. Reporting standards for functional diversity metrics are inconsistent across studies — some report Shannon and Chao1 indices, others report enzyme activities in varying units, and still others quantify functional-gene copy numbers via qPCR without harmonized normalization — impeding cross-study meta-analytic comparability and, in some cases, likely contributing to the apparent contradictions noted in Section 3.1 between studies reporting alpha-diversity gains and those reporting null alpha-diversity effects. Geographic representation is also skewed: the large majority of primary studies originate from temperate and subtropical Chinese agricultural systems, with comparatively few studies from tropical, heavily weathered, or smallholder-farming contexts in South Asia, sub-Saharan Africa, or Latin America, despite these regions representing priority targets for biochar-based soil restoration programs.

5.3. Emerging Technologies and Future Developments

Several methodological and technological trends are emerging that may address the gaps identified above. Multi-omics integration — combining metagenomics with

metatranscriptomics and metaproteomics — is increasingly used to move beyond static community composition toward realized functional expression, as exemplified by recent metagenomic functional-gene analyses of pyrolysis-temperature effects on carbon and nitrogen metabolic pathways [11]. Machine-learning and co-occurrence network approaches, as applied in the large-scale bacterial meta-analysis of Li *et al.* [9], represent a second emerging methodological frontier, enabling researchers to detect compositional and network-topological shifts that conventional alpha-diversity statistics fail to capture. Third, "designer" or engineered biochar — biochar deliberately modified or co-applied with functional microbial inoculants (AMF, *Bacillus subtilis*, and other plant-growth-promoting strains) to achieve targeted functional outcomes — is gaining traction as a precision alternative to generic biochar application [12, 18]. Finally, growing interest in biochar's role within formal carbon credit and monitoring-reporting-verification (MRV) frameworks is likely to drive demand for standardized, field-validated, longitudinal microbial functional data capable of underpinning credible carbon accounting claims.

6. Discussion

Taken together, the reviewed literature supports several integrated conclusions. First, biochar's modulation of soil microbial functional diversity is mediated through a coherent, if complex, causal chain: biochar properties (feedstock, pyrolysis temperature, pore structure) alter soil physicochemical conditions (pH, cation exchange capacity, nutrient availability), which in turn reshape microbial habitat and substrate availability, ultimately manifesting as measurable shifts in community composition, enzyme activity, and functional-gene abundance (Figure 3). This causal architecture is broadly consistent across the bacterial, fungal, enzymatic, and functional-gene sub-literatures reviewed in Section 3, even where the specific direction or magnitude of effects differs.

Second, the apparent contradictions that pervade this field — most notably, inconsistent alpha-diversity responses alongside consistent beta-diversity and functional shifts [7, 9] — are best explained not as genuine scientific disagreement but as a consequence of measuring different, only loosely correlated, dimensions of microbial community change. This has an important practical implication: agronomists and policymakers evaluating biochar's soil-health benefits should not rely on diversity indices alone as evidence of (or against) biochar efficacy, but should instead prioritize functional endpoints — enzyme activity, functional-gene abundance, and nutrient-cycling rates — that map more directly onto the ecosystem services of practical interest.

Third, the evidence robustly establishes that biochar's effects are not universal but are systematically moderated by pyrolysis temperature, feedstock, application dose, and soil context. This context-dependency, while scientifically well-documented, has not yet been translated into practical, region- and soil-specific application guidance for farmers and land managers, representing a significant gap between the maturity of the mechanistic evidence base and the maturity of implementation guidance. Fourth, the priming-effect literature reveals that biochar's carbon-sequestration claims require nuance: positive priming of native soil organic carbon is real, but appears to be a transient phenomenon that self-limits over several years as labile carbon is depleted or as organo-mineral

stabilization mechanisms take hold [26]. This finding is directly relevant to the growing use of biochar within voluntary carbon markets, where accounting frameworks should ideally incorporate multi-year, soil-specific priming dynamics rather than assuming static carbon behaviour.

Finally, this review's synthesis suggests that the field is at an inflection point: the foundational question of whether biochar affects soil microbial communities has been convincingly answered in the affirmative, and attention is now shifting toward the more actionable questions of how to engineer specific, predictable functional outcomes through precision feedstock and pyrolysis-temperature selection, and how to validate these outcomes over ecologically and economically meaningful timeframes. Realizing this shift will require the methodological advances discussed in Section 5.3 — particularly standardized reporting, multi-omics integration, and long-term field networks spanning currently under-represented soil types and geographies.

7. Conclusion

The investigation collected evidence from 98 peer-reviewed articles published between 2011 and 2026 to analyze the effects of biochar on microbial functional diversity in soil. The findings indicate that biochar is able to alter the physicochemical properties of the soil in a way that influences microbial composition, enzyme activities, and functional genes necessary for carbon and nitrogen cycles. However, specific outcomes might vary according to feedstock type, temperature of pyrolysis process, quantity, application method, soil condition, and time of application. While bacterial reactions are investigated better than fungal, archaeal, and protist reactions, there is a lot more short-term data from laboratory studies compared to long-term field studies.

For researchers, it is suggested that prioritizing functional diversity metrics that are standardized and harmonized as well as designing studies that allow for understanding the mechanisms of the effects of pH, supply of labile carbon, and habitat. In addition, the review suggests to increase the duration of the experiments in order to cover the processes of priming and stabilization for a couple of years and widen the geography and soils in studying functional diversity beyond temperate agricultural systems that dominate now. As for practitioners and policymakers, it seems reasonable to recommend cautious, context-specific biochar promotion rather than unified suggestion that application rates and relevant parameters may differ since the application of specific type of biochar should be in the line with each specific soil limitation and expected results, for example, while nutrient-rich biochar could be applied in degraded soils, one may require to employ high-temperature biochar that should serve the purpose of carbon stabilization rather than assume that the carbon efficiency of biochar would be the same in all cases.

Future studies would significantly gain from comprehensive, cooperative, multi-site, multi-year field networks that use streamlined multi-omics protocols, allowing the discipline to advance from a fertile but fragmentary evidence base to biochar-microbiome interaction predictive models based on specific soil and feed-stocks. These processes would assist in transferring the significant mechanistic knowledge that has already been achieved into efficient, scalable tools for effective soil management and climate mitigation policymaking.

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