Journal of Soil Science and Plant Nutrition
https://doi.org/10.1007/s42729-025-02650-2

REVIEW

Exploring Biochar Impacts Through Omics Sciences: Enhancing Rhizobiome Understanding

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Received: 4 November 2024 / Accepted: 28 July 2025

Abstract
Harnessing the potential of biochar in the rhizosphere to enhance plant health, growth and soil fertility is a promising avenue in agriculture. However, conventional research falls short in elucidating the underlying mechanisms of biochar's actions. Hence, the advent of multi-omics technologies becomes imperative in unravelling the multifaceted interplay among biochar, plants, and microbes within the dynamic rhizosphere. Metagenomics sheds light on microbial population dynamics following biochar application, while metatranscriptomics unveils gene expression and pathway regulation within microbial communities, offering insights into their metabolic intricacies. At the same time, metaproteomics and metametabolomics delve into protein products and metabolic profiles within the rhizobiome, respectively. Understanding the interactions of biochar with the rhizobiome holds promise in constructing predictive models and developing novel strategies to nurture soil health. This review focuses on using metaomics approaches to enhance biochar integration in agriculture, highlighting existing challenges in their application and emphasizing the need to overcome these barriers to improve soil fertility and microbial ecology and contribute to soil remediation.

Keywords Biochar · Rhizobiome · Metagenomics · Metatranscriptomics · Metaproteomics · Metametabolomics

1 Introduction

The diverse microbial community inhabiting a plant's root–rhizosphere interface plays a key role in determining and maintaining plant health (Castellano-Hinojosa & Strauss 2021; Olanrewaju et al. 2019; Orozco-Mosquedadel et al. 2022). Benefits to the plant from this intricate symbiosis include growth promotion, nutrient solubilization, stress amelioration, and disease resistance (Jamil et al. 2022). Meanwhile, the composition of root exudates secreted by plants shapes the rhizobiome, further influencing microbial abundance and diversity (Korenblum et al. 2022). Introducing soil amendments can induce shifts in microbial communities, modifying the rhizospheric soil chemistry and manipulating the rhizobiome to improve plant productivity (Mahmud et al. 2021). In this aspect, biochar generated from biomass pyrolysis has been investigated extensively in recent years for its soil-enhancing properties (Xu et al. 2023).

Biochar, a carbon-rich residue (Xie et al. 2023), originates from diverse biomass sources (Edussuriya et al. 2023; Haider et al. 2022; Kumar et al. 2023). Widely used in agriculture, biochar amendment demonstrates

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great potential in modifying soil physicochemical properties (Kumar et al. 2023; Xu et al. 2023), fostering sustainable crop production through improved soil fertility and enhanced nutrient uptake (Xu et al. 2023). Moreover, biochar can effectively remediate polluted soils (Li et al. 2020). However, unravelling the complex, multi-dimensional interactions among biochar, plants, and microbes in the dynamic rhizospheric zone demands more than conventional cultural approaches (Priya et al. 2021). Culture-independent molecular techniques are required to comprehensively analyze soil microbial communities and their underlying interactions (Joshi et al. 2023).

Recent developments in omics technologies have revolutionized our understanding of microbial signals from various plant interactions, elucidating physiological, metabolic, and biochemical responses (Qi et al. 2023). Metagenomics, a powerful interdisciplinary, culture-free tool, can characterize the genomic landscape of microbial communities within the rhizobiome, shedding light on structural and functional genomes (Ijaz et al. 2021). Metagenomic approaches have unveiled the potential of biochar to shape the microbial community composition in the rhizosphere (Kolton et al. 2017). Conversely, metatranscriptomics enables the characterization of actively transcribed functional genes within an ecosystem during sampling, providing insights into transcriptome profiles and microbial interactions through mRNA sequencing and microarrays (Vargas-Albores et al. 2019). Metaproteomics identifies the entire protein collection within an environmental sample at a specific time, facilitating a deeper understanding of microbial protein profiles, complex metabolic pathways, and multifunctional gene identification (Chiapello et al. 2020; Ijaz et al. 2021). The entire collection of metabolites within an ecosystem, termed the 'metametabolome,' (White et al. 2017a, b), transforms upon biochar addition, leading to changes in amino acid and organic acid levels and root exudates, which affect the rhizobiome and reshape the rhizosphere metabolome (Mohamed et al. 2021). Consequently, metametabolomics analysis unveils this altered metabolome, which offers insights into rhizospheric dynamics as moulded and modulated by biochar amendment (Sharma et al. 2021). Thus, multi-omics techniques such as genomics, transcriptomics, proteomics, and metabolomics as a package provide a holistic view of biochar-induced molecular changes within the rhizobiome (Fadiji et al. 2022), as depicted in Fig. 1.

Despite significant advances, understanding the complex, multidimensional interactions among biochar, plants, and rhizobiome remains a major challenge. Traditional methods fall short in capturing the dynamic holistic molecular processes involved. In this context, multi-omics technologies including metagenomics, metatranscriptomics, metaproteomics, and metametabolomics offer powerful, culture-independent tools to unravel the structural and functional complexity of microbial communities affected by biochar amendments.

This review aims to critically analyze recent findings on how multi-omics approaches have been used to investigate the effects of biochar on the rhizobiome. It highlights how these techniques reveal biochemical, physiological, and molecular changes that occur following biochar application, discusses current limitations and gaps in the literature, and advocates for integrative multi-omics frameworks in future studies. The significance of this review lies in its contribution to advancing sustainable agriculture by promoting a deeper, systems-level understanding of how biochar shapes soil microbial dynamics. This knowledge is crucial for optimizing biochar use to enhance crop productivity, soil health, and environmental resilience.

2 Bibliometric analysis on biochar effects on omics sciences in the rhizobiome

2.1 Method

Using information from the SCOPUS scientific database, we conducted a bibliometric analysis of peer-reviewed scientific literature on the 27th of February 2024, focusing on the impact of biochar on omics sciences in the rhizobiome. The search encompassed titles, abstracts, and keywords of publications in the Science Citation Index, employing specific keywords: ("biochar") AND ("omics" OR "metaomics" OR "multi-omics" OR "metagenomics" OR "metagenome" OR "genomics" OR "metatranscriptomic" OR "transcriptome" OR "metabolomic" OR "metametabolomic" OR "soil metabolites" OR "soil metabolome" OR "metaproteomic" OR "soil proteome") AND ("rhizobiome" OR "rhizosphere" OR "rhizomicrobiome" OR "soil microbiome"). Another search was conducted using the keywords ("biochar") AND ("rhizobiome" OR "rhizosphere" OR "rhizomicrobiome" OR "soil microbiome") for comparison. The search was confined to English research articles, excluding books, editorials, dissertations, and references published in languages other than English.

Of the 578 publications identified using the keywords ("biochar") AND ("rhizobiome" OR "rhizosphere" OR "rhizomicrobiome" OR "soil microbiome"), only 88 publications from 2013 to 2023 met the specified criteria (Fig. 2). The SCOPUS data indicate a significant increase in research papers on omics sciences applied to assess biochar effects on the rhizobiome since 2019 (Fig. 2). Using VOSviewer 1.6.20, we conducted a bibliometric analysis using keyword mapping to identify key study components. Keyword co-occurrence analysis offered valuable insights into interdisciplinary relationships, with 46 keywords meeting the criterion of appearing at least twice across all retrieved papers.

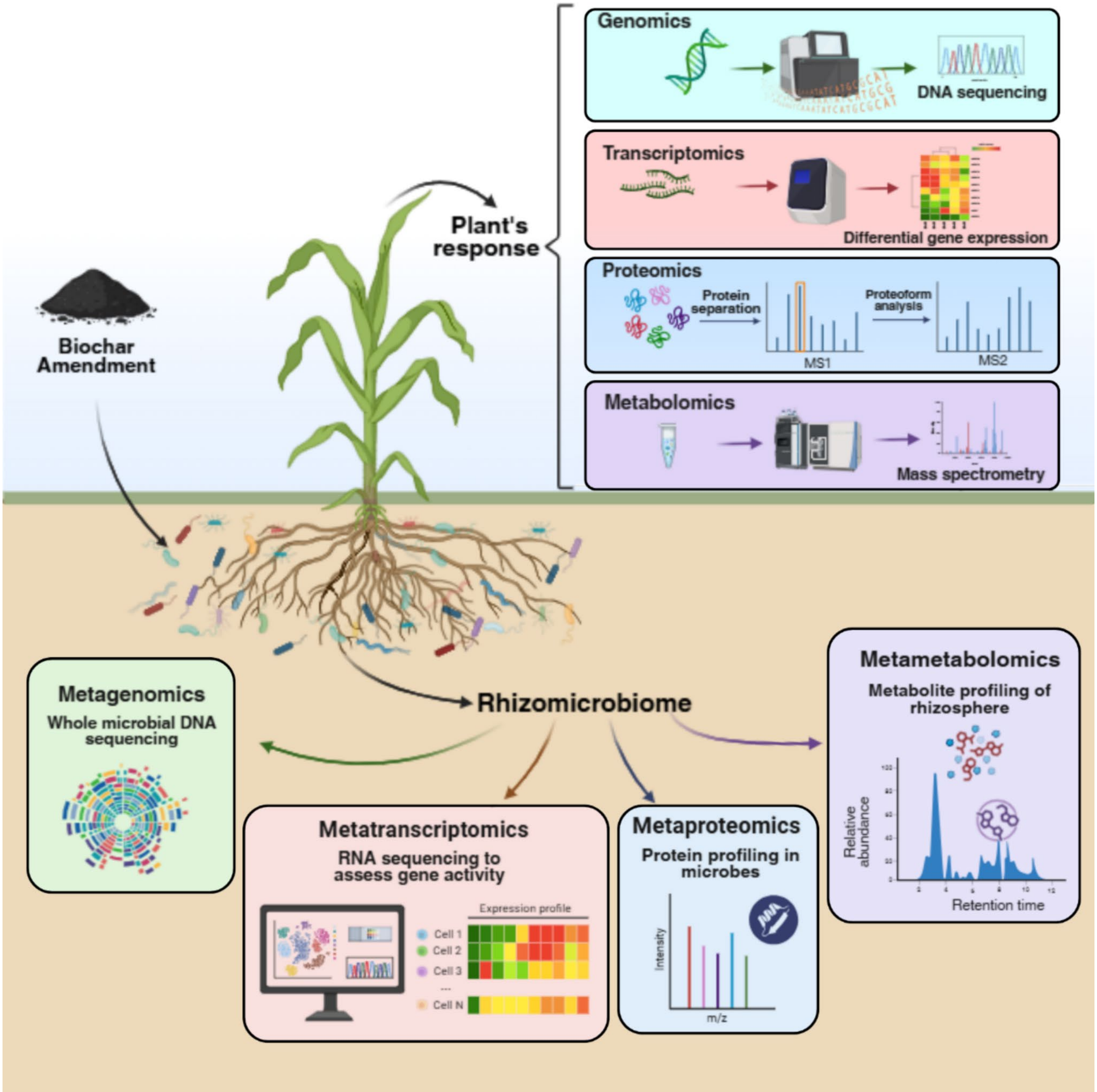

Fig. 1 Omics approaches for determining the effect of biochar amendment in soil

2.2 Results

Network visualization represents keywords as nodes with coloured edges, facilitating the identification of clusters and relationships. Overlay visualization compares keyword co-occurrence in different periods. Node size corresponds to keyword frequency in titles and abstracts. In the network map, larger nodes indicate higher frequency in analyzed documents, and thicker edges signify stronger relationships, with width reflecting connection strength, and weight indicating intensity.

Figure 3a depicts a keyword co-occurrence network with 43 nodes, revealing nine clusters based on frequent occurrences in selected publications. The term “Biochar” emerges as the most used author keyword, with 23 links and an average publication year of 2022. Clusters, such as red, green, and blue, stand out, with keywords like “transcriptome,” “metagenome,” and “metabolomics” garnering significant

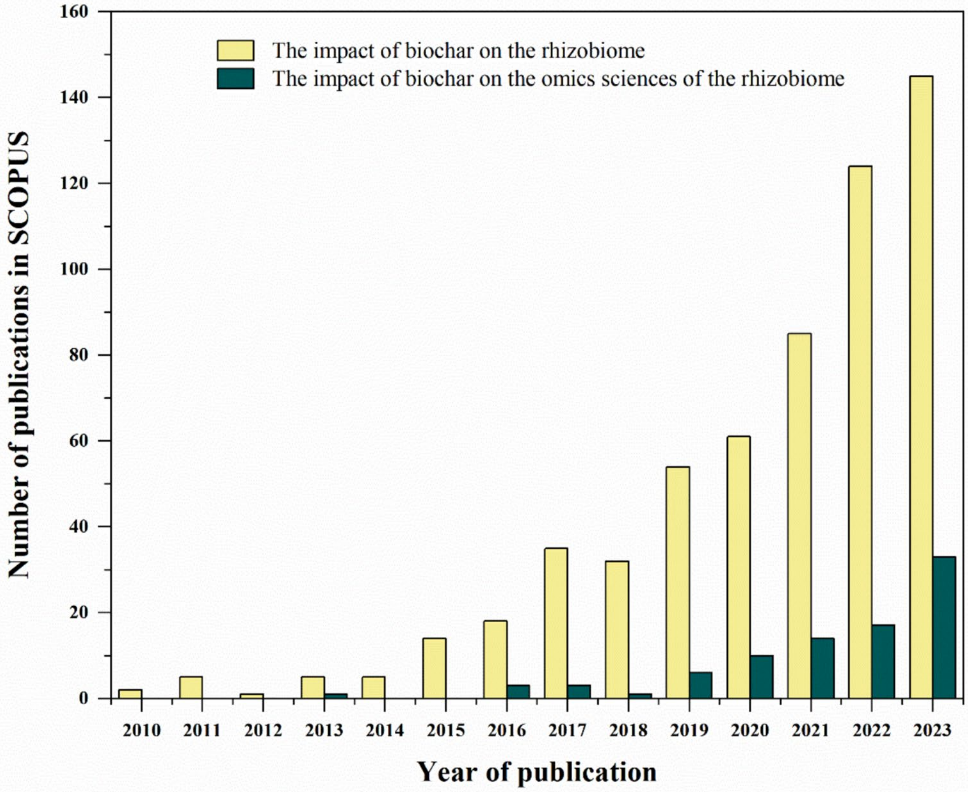

Fig. 2 Comparison of the number of publications in SCOPUS on the impact of biochar on the rhizobiome and that of the impact of biochar on the omics sciences of the rhizobiome

attention. The largest node, representing "biochar," boasts 39 co-occurrences, while "metagenomics" exhibits eight co-occurrences, notably tied to "biochar." Fig. 3B illustrates the temporal distribution of keyword co-occurrence in recent years. Nodes are colour-coded based on the average time of keyword appearance. Notably, transcriptomic studies are still emerging, while metagenomic studies are already available (Fig. 3b). Over the recent years, there has been a growing trend in using omics sciences to assess the biochar effects on the rhizobiome.

This analysis reveals a notable absence of key studies for understanding molecular-level changes in the rhizobiome following biochar amendment. This absence highlights the limited attention given to these crucial concepts and suggests unexplored avenues for future research. While the omics field has advanced significantly in assessing biochar effects on the rhizobiome since 2019, evident in the growing literature (Fig. 2), a closer inspection exposes a gap in keyword representation. Terms like metatranscriptomics and metaproteomics, vital for comprehending biochar effects on omics sciences in the rhizobiome, are underrepresented, raising questions about the comprehensiveness of current research, and emphasizing the need for further exploration in these areas.

3 Metagenomics to elucidate biochar-induced shifts in the rhizobiome

The rhizobiome comprises diverse microbial communities in the plant root system and surrounding soil, and plays a central role in ensuring soil fertility and plant health (Olanrewaju et al. 2019; White et al. 2017a, b). These plant-specific microbial communities are intricately shaped by the composition and abundance of root exudates released into the rhizospheric soil (Korenblum et al. 2022; White et al. 2017a, b). Consequently, biochar soil amendment, which improves plant nutrient uptake by altering soil physicochemical properties, influences microbial community dynamics (Haider et al. 2022). Traditional culture-based approaches fall short

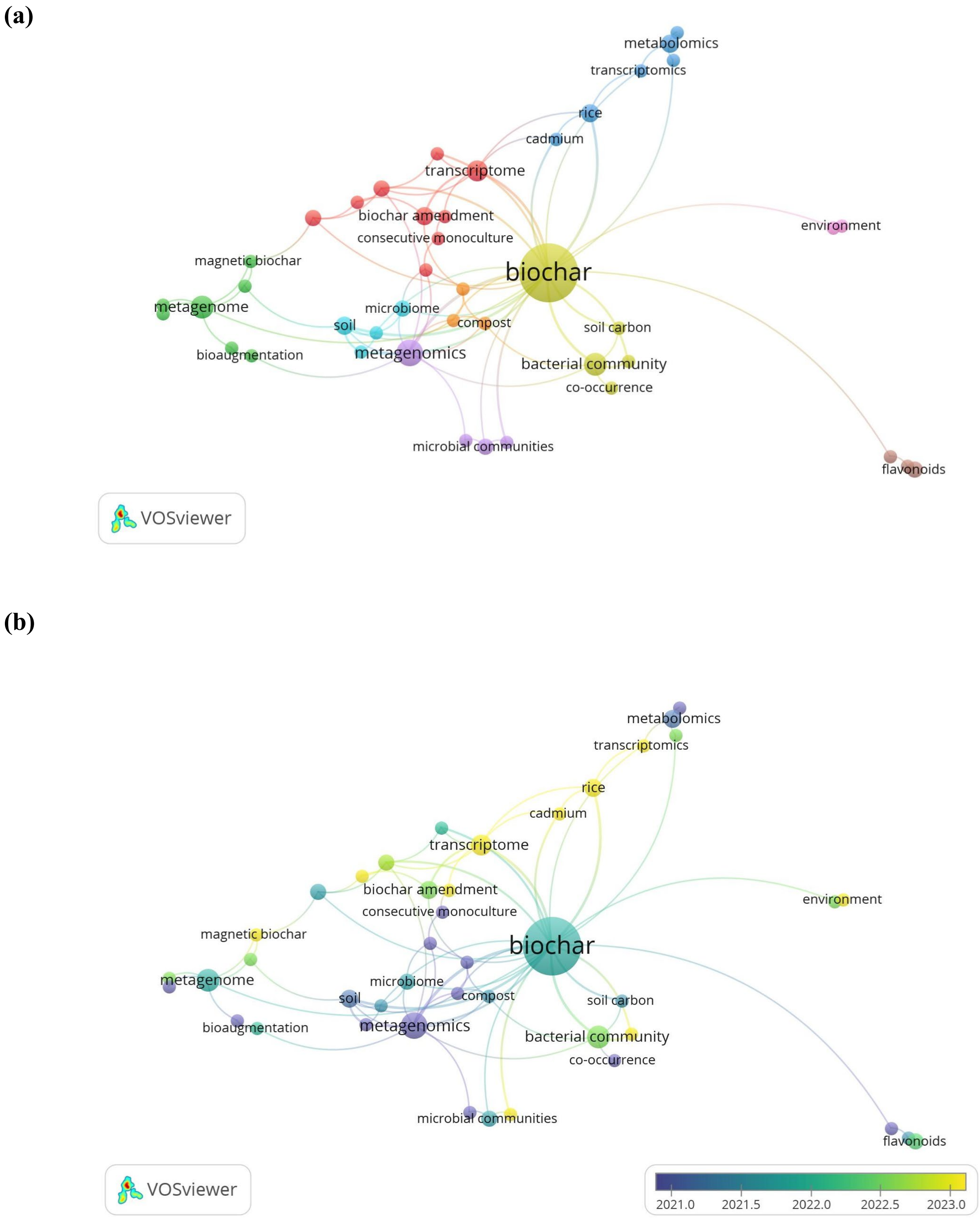

Fig. 3 (**a**) Network visualization and (**b**) overlay visualization of keyword co-occurrence of biochar effects on omics sciences in the rhizobiome

in capturing the full complexity of these microbial shifts (Acuna et al. 2020). Metagenomics, therefore, emerges as an indispensable tool to comprehensively profile rhizobiome communities and understand biochar-mediated transformations (Hewitt et al. 2023; Vargas-Albores et al. 2019).

3.1 Metagenomics approaches for characterizing rhizobiome diversity and function

Advances in molecular techniques have enabled metagenomics analyses to decipher microbial community diversity in the rhizosphere, even providing insights into the unculturable microbes (Priya et al. 2021). The initial step of a metagenomics strategy involves the collection of soil samples from biochar-amended rhizosphere soil for the extraction of DNA (Yang et al. 2025). Having sieved the moist soil to remove stones and roots, DNA extraction can be performed using a soil DNA extraction kit following the manufacturer's protocol (Gao et al. 2025). Then the extracted DNA is purified and quantified using a NanoDrop spectrophotometer and 1% agarose gel electrophoresis (Wu et al. 2025). To analyse the microbial diversity, specific primers and barcodes could be used to amplify the 16S rRNA and ITS gene regions (Wu et al. 2025). The resulting amplicons are sequenced and bioinformatic analyses are performed, thereby providing a broad picture of the microbial composition of the rhizobiome.

These sequence-based metagenomic studies have revealed microbial diversity patterns in response to biochar across plant genotypes and soil types. For instance, shotgun metagenomics and metagenomic analyses revealed the presence and abundance of 28 microbial phyla, including bacteria, archaea, and fungi, at various taxonomic levels in maize (*Zea mays*) rhizosphere soils (Omotayo et al. 2021). This study elucidated the correlation between the physicochemical properties of soil and the higher abundance of microbes with similar functions, unveiling the ecological roles of unknown microbial groups (Omotayo et al. 2021). Similarly, 16S rRNA gene amplicon sequencing and genome-resolved metagenomics of DNA extracted from the rhizobiome of two *Zea mays* genotypes — *Z. mays* ssp. *parviglumis* and *Z. mays* genotype B73 revealed that the core rhizobiome comprised Verrucomicrobiota, Proteobacteria, and Actinobacteria, and that the microbial communities of each genotype differed statistically (Yadav et al. 2023).

Beyond taxonomic resolution, functional metagenomics provides insights into microbial gene functions and metabolic pathways. While the above approach is referred to as sequence-based metagenomics, a function based metagenomics approach would be more beneficial in deciphering differential gene expressions and metabolic pathways associated with the plant–microbe interactions (Wani et al. 2024). This approach provides a microbial functional profile, indicating direct linkages between genes and microbial taxa (Wani et al. 2024). Lu et al. (2025) reported the functional annotation of non-redundant genes sequenced from DNA extracted from tobacco rhizosphere soil against the KEGG (Kyoto Encyclopaedia of Genes and Genomes) database which revealed a significant upregulation of the expression of Sulphur-metabolism related genes such as CysH and CysJ. This upregulation is hypothesized to enhance the synthesis of Sulphur-containing antimicrobial compounds to aid in pathogen suppression (Lu et al. 2025).

Thus, a metagenomics approach is indispensable for understanding the composition of and shifts in microbial communities in the rhizobiome as well as changes in the microbial functional profile with respect to the soil environment. This soil environment is ultimately governed by root exudates secreted by different plant genotypes and the recruitment of microbial taxa to roots.

3.2 Biochar-mediated modulation of rhizobiome composition and functional potential

Biochar's influence on the rhizobiome is context-dependent, shaped by biomass source, pyrolysis conditions, and application rate and soil conditions, cropping systems and contamination contexts as illustrated in Table 1 (Hewitt et al. 2023; Wu et al. 2022). These studies also vary in terms of DNA extraction methods, sequencing platforms, and bioinformatic pipelines used for taxonomic and functional profiling (Table 2). Such methodological diversity highlights the importance of experimental context when interpreting metagenomic data related to biochar–microbe interactions. A metaanalysis by (Wang et al. 2023) demonstrated that biochar derived from herbaceous materials or wood significantly ($p < 0.0001$) increased soil bacterial diversity compared to raw materials like domestic waste, lignocellulosic wastes, and manure, with optimal pyrolysis temperatures ranging from 350–550 °C (Wang et al. 2023). Moreover, Gao et al. (2024) reported differential effects of pristine corn (*Zea mays* L.) stalk biochar pyrolyzed at four different temperatures on the growth of *Microcystis aeruginosa*, a harmful cyanobacterium, with biochar pyrolyzed at 350 °C accelerating bacterial cell growth and at 550 °C inhibiting cell growth, which highlights the importance of pyrolysis conditions.

The impact of biochar on the rhizobiome can be elucidated by comparing microbial community structures with and without biochar treatment (Huang et al. 2023). For instance, biochar derived from rice hulls, generated at 500 °C, applied at 1 t ha^{-1} to the soil surrounding newly planted and one-year monoculture fields of *Radix pseudostellariae* increased the diversity and abundance of beneficial microbes such as *Bacillus, Streptomyces, Pseudomonas, Trichoderma*, *Mortierella* and *Acremonium* spp.,

Table 1 Biochar Treatments and Experimental Sites in Recent Metagenomic Studies on the Rhizosphere

Biochar Feedstock & Pyrolysis Conditions	Experimental Location	Cropping System/Soil Characteristics	References
Rice straw (pyrolyzed at 600 °C)	Kunshan Irrigation and Drainage Experimental Station in Taihu Lake Region, China	Rice–wheat rotation cropping system with potential for antibiotic-resistant soil contamination	(Zhang et al. 2024)
Orchard pruning feedstock (pyrolyzed at 500 °C)	Vineyard "La Braccesca Estate" Montepulciano (Tuscany, Central Italy)	Vineyard with acidic soil (sandy, clay loam texture)	(Idbella et al. 2024)
Rice hull (pyrolyzed at 500 °C)	Zherong City, Fujian Province, China	Rhizosphere soil of *R. pseudostellariae* in a field consecutively planted for two years	(Yan et al. 2024)
Bamboo (pyrolyzed at 800 °C)	Yellow River Delta, China	Surface soil in an area rich in nearby oil resources (phenanthrene-contaminated soil)	(Lu et al. 2024)
Pinecone-derived biochar and its six modified biochars: (amino, epoxy, ethoxy, thiol, selenium, chitosan)	Suburbs of Beibei, Chongqing, China	Soil from rice paddy field contaminated with Hg	(Guo et al. 2024)
Water hyacinth (*Eichhornia crassipes*) (pyrolyzed at 500 °C)	Rangsit, Pathum-Tani district, Thailand	Soil from Klong farmland	(Jutakanoke et al. 2023)
Rice straw (Heated at 160 °C with relevant ingredients to form magnetic biochar, and then pyrolyzed at 700 °C)	Grumolo delle Abbadesse (VI, Italy)	Soil from paddy field	(Ji et al. 2023)
Wheat straw (pyrolyzed at 500 °C)	Northwest A&F University in Yangling, Shaanxi Province, China	Semi-arid agricultural area containing anthrosol soil	(Li et al. 2023)
Rice straw (pyrolyzed at 550 °C)	Agricultural field site at an organic agriculture demonstration station, Suzhou City, Jiangsu Province, China	Typical DEHP-contaminated area	(Ortiz-Liébana et al. 2023)
Vine shoots (*Vitis vinifera*) (pyrolyzed at 550 °C)	Los Lorentes Rambla Salada	Melon and pepper fields	(Ortiz-Liébana et al. 2023)

while decreasing the relative abundance of Zixibacteria, Nitrospirae and pathogenic fungi, such as *Fusarium* sp. and *Talaromyces* sp. (Wu et al. 2022*)*. Another study, using 16S and 18S amplicon metagenomics to characterize the rhizobiome of *R. pseudostellariae* consecutively cultivated in a two-year monoculture field amended with 1 t ha^{-1} rice-hull biochar, further revealed how biochar addition significantly influenced rhizobiome composition, favouring potentially beneficial *Bacillus* sp. while suppressing pathogenic *Fusarium oxysporum* (Yan et al. 2024). These findings underscore the role of metagenomics in understanding biochar-mediated shifts in microbial communities and their implications for biocontrol.

Insight into rhizobiome changes can advance our understanding of the effect of different biochar types on beneficial and pathogenic microbes (Diwan et al. 2022), facilitating tailored biochar formulations. However, factors such as biomass type, pyrolysis temperature, biochar formulation, biochar exposure time, host plant species, soil matrix, study location or site, and study duration can influence the specific effect of biochar on the rhizobiome, complicating the generalization of biochar's effect on the rhizobiome.

The challenge in pinpointing the exact response of microbial communities to biochar amendment stems from the contrasting pathways observed in certain scenarios. For instance, a metagenomic analysis combined with metabolomics revealed that 2% (w/w) bamboo biochar applied to soil contaminated with phenanthrene (PHE), a polycyclic aromatic hydrocarbon (PAH), inhibited PHE degradation (Lu et al. 2024), despite biochar's recognized role in PAH-contaminated soil remediation (Li et al. 2020). This inhibition was associated with a reduction in bacterial genera containing cytochrome P450 monooxygenase (CYP116) due to biochar, while the high C/N ratio of the biochar treatment hindered the growth of PHE degraders. Conversely, the same study found that soil amended with 2% (w/w) bamboo biochar inoculated with a *Pseudomonas putida* suspension enhanced PHE degradation, attributed to the increased abundance of cytochrome P450 superfamily CYP116, despite potential negative effects on PHE degraders (Lu et al. 2024). Consequently, published studies present conflicting evidences, indicating a lack of consensus about pattern of microbial response to biochar amendment, particularly when contrasting pathways arise due to formulation changes.

Table 2 DNA Extraction, Sequencing Platforms, and Bioinformatic Pipelines Used in Recent Rhizosphere Metagenomic Studies Involving Biochar

DNA Extraction Method	Sequencing Platform	PCR Target Region	Bioinformatics Tools & Databases	References
Mag-Bind® Soil DNA Kit (Omega)	Illumina NovaSeq 6000 platform	NI	ARG and MGE identification and quantification: CARD and NCBI databases using DIAMOND (v0.8.22) Bacterial classification: NCBI bacterial reference sequences using Kraken2 (v2.0.7-beta) Species abundance: Bracken (v2.0)	(Zhang et al. 2024)
DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany)	Illumina MiSeq platform	V3–V4 region of bacterial 16S rRNA gene and ITS1–2 region	UNITE database for fungi	(Idbella et al. 2024)
Soil DNA Extraction Kit (BioFast, China)	Illumina NovaSeq PE250 sequencer	18S rRNA gene	High-quality sequences analysis: QIIME2 Taxonomic assignment: SILVA database	(Yan et al. 2024)
Fast DNA Spin Kit (MP, USA)	Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA)	*CarA* gene -*Pseudomonas putida* PAH ring-hydroxylating dioxygenase (PAH-RHD) genes	Gene abundance: SOAP aligner (soap2.21) NCBI non-redundant database Microbial community structure and functional annotation: KEGG database using Diamond (v.2.0.13) and CYP450 Database	(Lu et al. 2024)
Fast PrepDNA Isolation Kit (MpBio, USA)	Illumina MiSeq PE300 platform (Illumina, San Diego, USA)	V4 region of bacterial 16S rRNA gene	OTUs clustering: UPARSE version 7.1 Taxonomy of each OTU representative sequence: 16S rRNA database (Silva v138) by RDP Classifier version 2.2	(Guo et al. 2024)
QIAamp PowerFecal Pro DNA Kit	MiSeq	V3–V4 region of 16S rRNA gene	Taxonomic classification: SILVA database	(Jutakanoke et al. 2023)
DNeasy PowerSoil (QIAGEN GmbH, Hilden, Germany)	Illumina NovaSeq platform MinION device (Oxford Nanopore Technologies, Oxford, UK)	NI	Taxonomic assignment of MAGs: GTDB Gene prediction in metagenomic data: Prodigal (v2.6.2) Associated genes with biological pathways and functions: KEGG database using EggNOG (v2.0.1–1) and DIAMOND (v0.9.22)	(Ji et al. 2023)
OMEGA Soil DNA Kit (D5625-01) (Omega Bio-Tek, Norcross, GA, USA)	Illumina NovaSeq platform at Shanghai Personal Biotechnology Co. Ltd (Shanghai, China)	V5–V7 region of 16S rRNA gene	Microbiome bioinformatics analyses: plugin-based QIIME2 2019.4 system	(Li et al. 2023)
PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA)	Illumina MiSeq platform Magigene Technology Co., Ltd (Guangdong, China)	V4–V5 region of 16S rRNA gene	RDP classifier (v. 2.2) algorithm in SILVA database	(Luo et al. 2023)
DNeasy Power Soil kit (Qiagen, Hilden, Germany)	Illumina MiSeq high-throughput sequencing platform at MR DNA	V4 region of 16S rRNA gene	NCBI	(Ortiz-Liébana et al. 2023)

ARG antibiotic resistance genes, *DEHP* di−(2−ethylhexyl) phthalate, *MGE* mobile genetic elements, *PAH* polycyclic aromatic hydrocarbon, *PAH−RHD* PAH ring−hydroxylating dioxygenase, *KEGG* Kyoto Encyclopedia of Genes and Genomes, *OTU* Operational taxonomic units, *MAG* metagenome−assembled genomes, *GTDB* Genome Taxonomy Database, *MR DNA* molecular research DNA, *NCBI* National Centre for Biotechnology Information

Recent literature focused on analysing the microbial pathways involved in PAH degradation have elucidated the stimulatory effect of low molecular weight organic acids (LMWOAs) such as citric, lactic, oxalic and glutaric acids excreted by plant root exudates (S. Zhang et al. 2021a, b) as well as microorganisms (Kohlmann et al. 2025). While LMWOAs generally contribute to microbial growth and activity, thereby promoting the efficiency of PAH degradation (L. Zhang et al. 2021a, b), there is a lack of clarity regarding the interactions between biochar and LMWOAs. While studies suggest that these variations in biochar effects may be attributed to the different pyrolysis temperatures, ageing and rate as well as duration of addition of biochar, there is a lack of comprehensive explanations of biochar's mechanisms, emphasizing the need for further research (Kohlmann et al. 2025).

Contrasting effects of biochar have also been documented, with the same formulation affecting different microbial communities in distinct ways. For instance, applying sugarcane straw biochar generated at 550–650 °C to sugarcane ratoon fields enriched biocontrol agents such as *Pseudomonas, Weissella*, *Pantoea,* and *Bacillus* and depleted genera like *Mycobacterium* and *Enterococcus* (Fallah et al. 2023). Furthermore, soil microbial metagenomics analysis revealed significant adverse effects ($p < 0.05$) of high-dose application of 5% peanut hull and straw biochar on soil bacterial and archaeal communities, with no significant effect on soil fungal communities (Zhu et al. 2023a).

In summary, these findings highlight the need for extensive research as well as the lack of a standardized protocol for biochar production, as there have been instances where the same biochar type led to differing and unpredictable changes in microbial composition and functional profiles. While it is understood that different factors may be attributed to these discrepancies as discussed before, the introduction of a standardized protocol could be utilized for fair and consistent comparison of microbial composition shifts when the same type of biochar is used for the same plant species or genus. This may involve the pyrolysis of the raw material at a specific temperature and a defined concentration of biochar formulation for soil amendment. The period of exposure to the biochar amendment is also a governing factor in the experimental design of these studies and therefore, an appropriate exposure period must also be selected for an optimized protocol. Furthermore, guidelines will have to be introduced for sample collection and DNA extraction. Such standardization will thus streamline the metagenomics analyses, enhancing the reliability and generalizability of findings.

4 Functional insights into plant–microbe interactions via metatranscriptomics

Metatranscriptomics has become a pivotal tool for unravelling the complex dynamics of the plant microbiome, particularly within the rhizosphere, revealing microbial diversity and elucidating the intricate network of gene interactions within this community (Varma et al. 2020). Unlike metagenomics, which only identifies potential pathways, microbial diversity, and genes within an ecosystem without distinguishing active components, metatranscriptomics provides insights into the functional capabilities and regulatory responses of live microbial systems (Kar et al. 2021; Nath et al. 2021). This enables researchers to distinguish not only who is present, but which microbial genes are actively functioning under specific soil and root conditions (Gonzalez et al. 2018; Thijs et al. 2016). The rhizosphere, the soil region immediately in contact with plant roots, undergoes constant modulation through the rhizo-deposition of mucilages, exudates, and sloughed plant cells (Nath et al. 2021). As a result, plant roots impact the surrounding soil and its inhabiting microbiome, while the rhizobiome reciprocally influences plant growth and productivity by producing plant growth regulatory compounds (Philippot et al. 2013; Spence et al. 2014). Thus, unravelling the underlying molecular interactions and elucidating the cross-kingdom, bi-directional communication channel between the plant and its rhizobiome requires the application of metatranscriptomics (Peng et al. 2024).

4.1 Biochar-induced transcriptional responses in rhizobiomes and host plants

The integration of plant transcriptomics with metaomics technologies is crucial for unravelling molecular intricacies in plant–microbe interactions, especially concerning the effects of biochar. Although root exudates play a key role in shaping rhizobiome composition, the complex interplay between plant development, exudation patterns, and microbial community assembly remains poorly understood (Kar et al. 2021). Metatranscriptomics addresses this gap by enabling the analysis of actively expressed microbial genes in response to plant-derived compounds. This process involves extracting total RNA from rhizosphere soil, enriching for polyadenylated mRNA, synthesizing complementary DNA (cDNA), and constructing cDNA libraries for high-throughput sequencing (Ajith Kumar & Ravi 2024; Pandiyan et al. 2022). The resulting sequence data are processed through bioinformatic pipelines to identify differentially expressed genes (DEGs) and active microbial functions. Functional annotation and network analysis further illuminate key microbial pathways affected by biochar or root exudates. When integrated with other omics approaches, metatranscriptomics offers a more holistic understanding of rhizospheric microbial responses to plant cues and soil amendments.

For instance, inoculation of *Populus trichocarpa* with *Mortierella elongata* upregulated genes related to lipid signaling, nutrient uptake, and gibberellin signaling, promoted plant growth, and modulated plant defense responses (Liao et al. 2019). Moreover, comparative metatranscriptomics revealed significant upregulation in *M. elongata* for RNA modification, translation, signal transduction, lipid transport, metabolism, and chitinase when co-cultured with *Populus versus* in pure culture (Liao et al. 2019). A heterospecific plant bioassay experiment growing *Pinus taeda* and *Populus trichocarpa* in different combinations used comparative metatranscriptomics data generated from 4-month-old root samples to determine in situ plant–microbe interactions and gene expressions in the root microbiome (Liao et al. 2021). While the rhizobiome of each plant species demonstrated high host-specificity with a distinct diversity of microbial taxa, the results also revealed microbial shifts and changes in microbial gene expression of the root microbiome (Liao et al. 2021). Moreover, nearby plants modifying the rhizobiome could regulate fundamental plant physiological processes by influencing molecular functions within the rhizobiome (Liao et al. 2021). Thus, specific rhizobiomes have been implicated in modulating gene expression in plants, affecting the plant transcriptome and metabolic profiles, in a phenomenon referred to as "systemically induced root exudation of metabolites" (SIREM), as portrayed in Fig. 4 (Korenblum et al. 2020). Modulating one plant's gene expression by another plant host via the mediation of the rhizobiome in this phenomenon has been recognized as a root–root long-distance signaling mechanism (Korenblum et al. 2022).

On the other hand, studies have also reported using transcriptomics to understand the effect of biochar amendment on plants. Transcriptome profiling of fruit and leaf samples collected from the pepper (*Capsicum annuum* L.) variety *Quijiao No.1* grown on soil infested with *Fusarium oxysporum* f. sp. *capsici* and amended with 100 kg mu^{-1} of bamboo biochar revealed DEGs involved in multiple pathways, such as protein processing in the endoplasmic reticulum, photosynthesis and its related pathways, carbon metabolism, plant-pathogen interaction, and antigen processing and presentation (Zhu et al. 2021). Since antigen processing and presentation pathways are associated with immune responses, these findings suggest that biochar amendment improves plant nutrition and enhances disease resistance to *Fusarium* wilt disease in pepper (Zhu et al. 2021).

Additionally, transcriptome sequencing of the roots of Chinese cherry 'Manaohong' (*Cerasus pseudocerasus* Lindl.) to determine its response to 3% tobacco straw biochar revealed the differential expression of 1,829 genes and biochar's involvement in activating a pathway associated with plant growth promotion (Yang et al. 2022). Upregulation of flavonoid biosynthesis further supported biochar's effect in shaping the rhizobiome by influencing root exudation and thus increasing plant growth-promoting bacteria, such as *Sphingomonas* sp. (Yang et al. 2022). However, the metagenomic analysis also showed a decrease in bacteria involved in nitrogen cycling due to competition for nitrogen between the rhizobiome and the Chinese cherry plant upon biochar addition, a pivotal insight into understanding that the upregulation of genes related to plant nitrogen uptake decreased the abundance of bacteria involved in nitrogen cycling (Yang et al. 2022). A transcriptomic analysis of peanut roots (*Arachis hypogaea* L. variety, 'Hua Yu 22') revealed that 2% peanut shell biochar improves root development and promotes oxidative stress resistance, nitrogen transport, and energy metabolism in response to microplastics contamination-related genes, while metagenomics applied to the rhizobiome demonstrated that biochar application restored the richness and diversity of microbial communities related to nitrogen cycling and organic matter decomposition (Yang et al. 2024).

While plant transcriptomics provides a holistic view of differentially expressed functional genes in plants, and metagenomics assesses the genetic potential of a microbial community, the presence of microbial residues can influence metagenomics. Thus, the significance of using a metatranscriptomics approach becomes evident, facilitating the correlation between the influence of biochar on plants and its ensuing effects on plant–microbe interactions in the rhizobiome. This approach aids in identifying viable microbes and the functional genes expressed during sampling.

4.2 Functional insights and limitations of metatranscriptomic approaches in biochar studies

Plant growth stimulation observed upon biochar amendment of soil can be attributed to the differential gene expression of the rhizobiome and plant roots (Hewitt et al. 2023; Martínez-Gómez et al. 2022). Thus, the importance of a metatranscriptomics approach in obtaining a comprehensive gene expression profile of the rhizobiome, along with the transcriptomic response of the plant, for deciphering the underlying molecular and physiological mechanisms of biochar mediation is evident (Jaiswal et al. 2020). Metatranscriptomic analyses have been instrumental in acquiring comprehensive data on microbial community adaptation and metabolic response upon biochar addition. For instance,

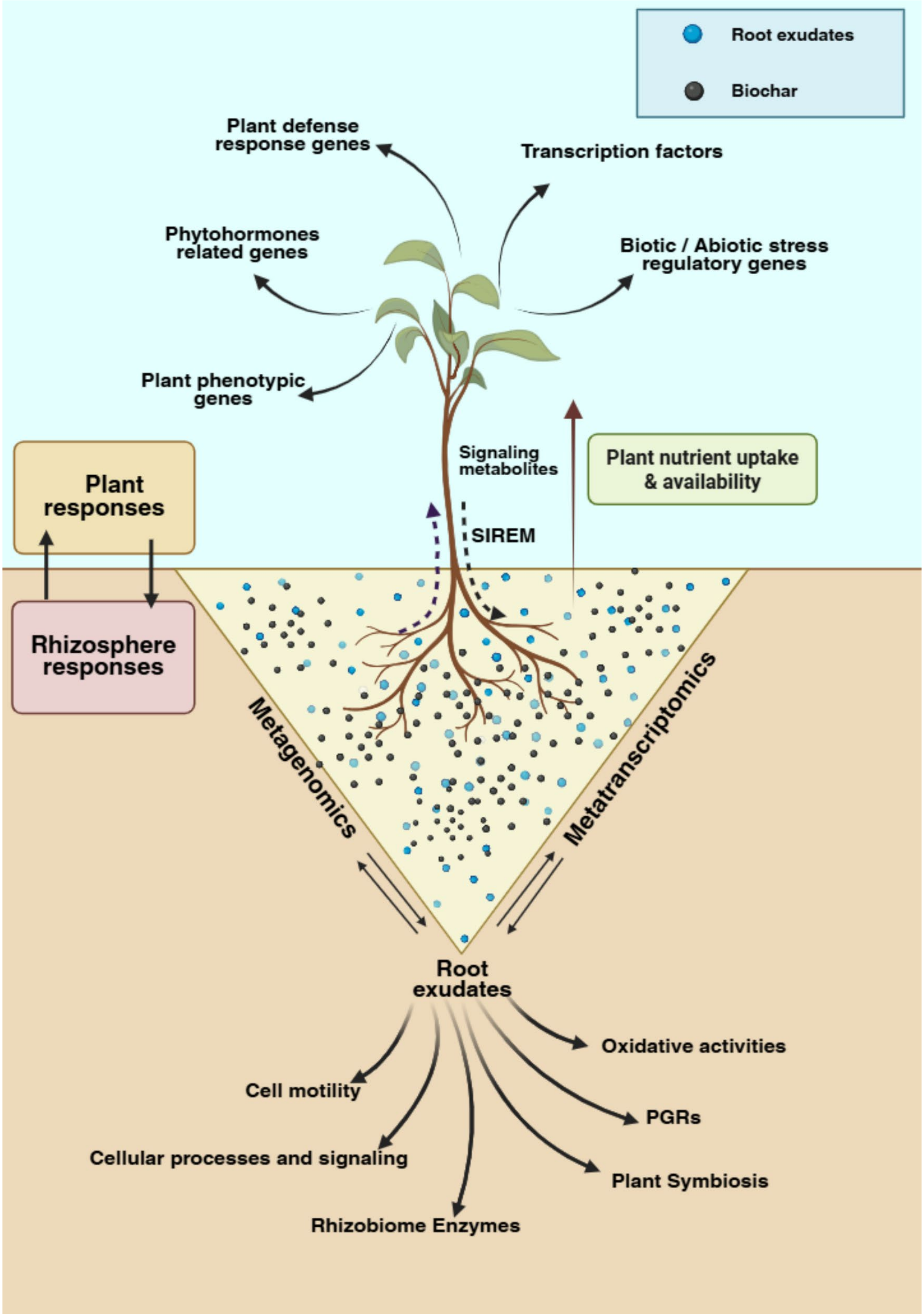

Fig. 4 Integration of plant transcriptomics with rhizobiome metaomics

metatranscriptome analysis of the rhizobiome of tomato (*Solanum lycopersicum* L.) in response to organic-certified biochar revealed elevated microbial gene expression compared to the control, which increased the abundance of bacteria beneficial for soil health and plant growth by enhancing functions such as nitrogen fixation, nitrogen and carbon cycling, plant growth promotion, organic compound biodegradation, and disease suppression (Hewitt et al. 2023). Gene Ontology enrichment analyses conducted for the differentially expressed genes of microbial communities and plant roots in the biochar-amended rhizosphere thus revealed the enrichment of nitrogen compound-associated processes, thereby supporting biochar influence in plant growth promotion through an increase in the nitrogen-fixing bacteria (Hewitt et al. 2023). Therefore, rather than focusing solely on shifts in microbial abundance, this approach highlights the effectiveness of the soil amendment by revealing the role of biochar in plant growth promotion.

Genome-centric metatranscriptomics further refines the focus to specific microbial genomes within the community, allowing the exploration of metagenome-assembled genomes (MAGs). For example, genome-centric metatranscriptomics demonstrated that hydrochar prepared from corn straw promoted anaerobic digestion of waste-activated sludge by enriching *Syntrophomonas* sp. FDU0164 and *Methanosarcina* sp. FDU0106, involved in direct interspecies electron transfer and methanogenesis (Shi et al. 2021). Similarly, in another study, genome-centric metatranscriptomics elucidated the role of pyrochar obtained at 500 °C in the competition between iron reduction and methanogenesis in an anaerobic digestion system by identifying the upregulation of out membrane cytochrome c and pilA in *Geobacter soli* A19, a dissimilatory iron-reducing bacterium (Cheng et al. 2023). Thus, mapping metatranscriptomics mRNA to assembled metagenomes aids in determining MAG activity and provides insights into the dynamics of complex plant–microbe–biochar interactions.

In a recent study reported by Gao et al. (2024), the effect of corn straw biochar (pyrolyzed at 450 ºC) on continuously cropped pepper plants was investigated. In line with previous findings from similar studies on the effects of biochar amendment, DNA extraction, sequencing and bioinformatic analyses of pepper rhizosphere soil samples confirmed a significant effect of biochar on the structure and distribution of the pepper rhizobiome across different developmental stages of the plants (Gao et al. 2024). Interestingly, biochar-induced microbial shifts strongly correlated with the soil electrical conductivity (SEC) (Gao et al. 2024). The SEC was influenced by biochar treatment, as it provides microbial habitats with high porosity (Gao et al. 2024) due to the porous structure and irregular shape which favour soil microbes and water storage (Yang et al. 2024). Thus, biochar-induced shifts in the SEC led to notable changes in bacterial genera such as an increase in *Steroidobact*er, involved in PAH degradation and heavy metal absorption and a decrease in *Bryobacter* (Gao et al. 2024), involved in nitrate reduction, thereby favouring the nitrate accumulation in soil (He et al. 2023). Moreover, the functional annotation of the bacterial genes revealed that they were involved in carbohydrate metabolism, terpenoid metabolism, polyketide metabolism, amino acid metabolism, genetic information processing, cellular processes and environmental information processing (Gao et al. 2024). Biochar treatment was also found to enhance the expression of glycolysis and Co-A biosynthesis-related genes and saturated fatty acid elongation-pathway related genes in the fungi of the rhizobiome (Gao et al. 2024).

However, despite its potential to unveil underlying molecular interactions, the application of metatranscriptomics for deciphering the role of biochar in the rhizobiome remains largely neglected (Peng et al. 2024). The success of the metatranscriptomics approach relies heavily on the quality and yield of mRNA extracted, which is hindered by factors such as the short half-life and low yield of mRNA, high amounts of impurities, and the heterogeneity of the rhizosphere (Peng et al. 2024). Moreover, interference by plant RNAs and signaling molecules involved in cross-kingdom communication between the plant and the rhizobiome further complicates the process (Peng et al. 2024).

5 Metaproteomics to unveil biochar's effect on the rhizobiome

While metagenomics portrays the microbial diversity of the rhizobiome and their shifts due to biochar amendment, metaproteomics offers a comprehensive perspective on the influence of biochar on the rhizobiome by revealing the protein profile of the rhizospheric sample (Abiraami et al. 2020). Unlike metatranscriptomics, metaproteomics benefits from the longer half-life of proteins, enabling exploration of the functional microbial communities in the rhizobiome (Abiraami et al. 2020). Metaproteomics involves several key steps: sample collection and preparation, protein separation, peptide analysis, protein identification, and data interpretation (Nebauer et al. 2024). The process begins with soil protein extraction through cell lysis, which can be achieved using chemical, mechanical, thermal, or enzymatic methods (Nebauer et al. 2024). Extracted proteins are then purified and separated using techniques such as sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional PAGE (2D-PAGE) (Pan et al. 2024). The resulting peptides are analyzed by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS), and bioinformatic search algorithms match the peptide spectra against protein databases to identify proteins (Pan et al.

2024). Finally, proteins are assigned taxonomic origin and functional roles through annotation pipelines. While specific protocols may vary depending on the sample type and research objectives, these steps form the foundation of the metaproteomics workflow.

This approach efficiently predicts ecosystem functioning and identifies active microbial taxa (Starke et al. 2022), providing crucial insights into tripartite (biochar–plant–rhizobiome) interactions (Tartaglia et al. 2020). Given the importance of root exudates in microbe recruitment and microbial community shaping in the rhizosphere (Afridi et al. 2023), metaproteomics can elucidate microbial shifts in response to carbon addition from biochar amendment. Root exudates rich in amino acids support microbe-mediated nitrogen mineralization in the rhizosphere (Lidbury et al. 2021). Therefore, the enrichment of proteins in the rhizosphere due to biochar amendment reflects changes in plant–microbe interactions. Moreover, biochar amendment influences rhizosphere enzyme activity, an indicator of the intensity of biological processes in the rhizosphere (Fan et al. 2023). Metaproteomic analyses can thus reveal enzyme activity changes, reflecting shifts in core functional microorganisms responding to soil nutrient shifts due to biochar amendment.

Despite its potential, there remains a dearth of studies focusing the effect of biochar on the metaproteome. Challenges such as soil matrix complexity and variability (Tartaglia et al. 2020), interference from contaminants (e.g., humic acids, polyphenols, and vertebral keratin) (Abiraami et al. 2020; White III et al., 2017), and low protein coverage (Tartaglia et al. 2020) hinder metaproteomic analyses. Thus, utmost care must be taken when obtaining a representative and consistent soil sample, ensuring a minimal time gap between collection and storage in a buffer for protease deactivation (Pan et al. 2024). The protocol will have to be modified accordingly to incorporate protease inhibitors as well as strong alkalis to recover proteins without humic substances (Pan et al. 2024). There is also the possibility of obtaining different protein yields when multiple replicates are involved and these discrepancies could hamper the subsequent analyses, leading to variable and unreliable results. Moreover, the complex, biochar- and species-specific nature of biochar–plant–rhizobiome interactions demands expertise in protein extraction and cell lysis (Tartaglia et al. 2023). Hence, further research and development including the incorporation of sophisticated protocols, instrumentation and advanced bioinformatic tools are needed to overcome these challenges and enable a comparative metaproteomics approach to understand the impact of biochar on functional microbial communities in the rhizobiome.

6 Metametabolomics to unveil the effect of biochar on the rhizobiome

Metametabolomics emerges as a vital tool for unravelling the intricate interactions between biochar, the rhizobiome, and soil biogeochemical cycles. By delving into soil metabolomics, this approach offers insights into rhizosphere dynamics following biochar amendment (Ijaz et al. 2021). Biochar addition triggers plants to secrete secondary metabolites in response to soil environmental changes (Cheng et al. 2018), influencing microbe recruitment and shaping of the rhizobiome. Biochar also alters soil composition, impacting microbial community diversity (Lu et al. 2024). Moreover, the rhizobiome reciprocally influences the root exudation of metabolites involved in recruiting microbes and shaping the rhizobiome (Afridi et al. 2023) due to a phenomenon known as SIREM (see Sect. 4.1; (Korenblum et al. 2020). Given the complexity of these four-way interactions, illustrated in Fig. 5, successfully implementing a metametabolomics approach becomes crucial for comprehensively understanding the effects of biochar amendment on the rhizosphere.

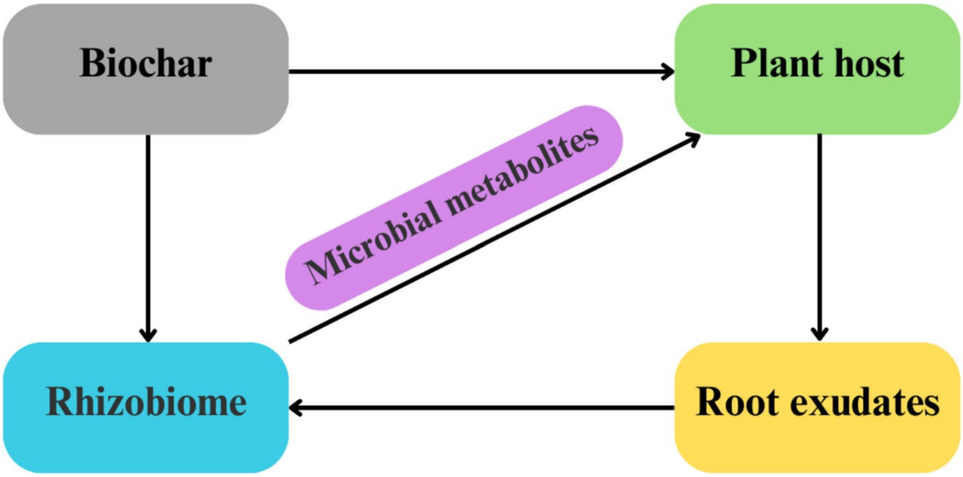

Fig. 5 Four-way interactions among plant host, root exudates, and the rhizobiome in response to biochar amendment of soil

6.1 Application of metametabolomics

Metabolites, the final products of cellular activities, offer crucial insights into how plants respond to various environmental stresses, both biotic and abiotic ones (Zhu et al. 2021). With over 200,000 different metabolites synthesized by plants for maintaining cellular integrity and regulating physiological processes (Singha et al. 2022), metabolomics provides a powerful tool for their detection, identification, and quantification (Singha et al. 2022). This involves rhizosphere soil collection, homogenization of the samples, metabolite extraction using appropriate solvent mixtures and metabolic profiling of the concentrated extracts (Hildebrand et al. 2023), using techniques such as LC–MS, Gas chromatography-mass spectrometry (GC–MS) and nuclear magnetic resonance (NMR), followed by the comparison of spectra obtained against databases such as KEGG (Korra et al. 2023). By employing mass spectrometry (MS) and NMR techniques for metabolic profiling of the rhizosphere, researchers can gain valuable insights into the metabolites secreted by plants and the rhizobiome, shedding light on their metabolic and functional roles in the ecosystem (Qi et al. 2023; White et al. 2017a, b), which are depicted in Fig. 6. Table 3 shows how metametabolomics analysis can be fundamental in deciphering the specific roles and functions of the rhizobiome, aiding in the understanding of a plant's species-specific response to varying soil physicochemical characteristics. However, it is essential to integrate the metametabolomics approach with metagenomics to fully elucidate the functional microbial communities in the rhizobiome and their roles in promoting plant growth and suppressing pathogens. This integration is necessary due to the significant influence of root exudates on the rhizosphere metabolome, which subsequently shapes the composition

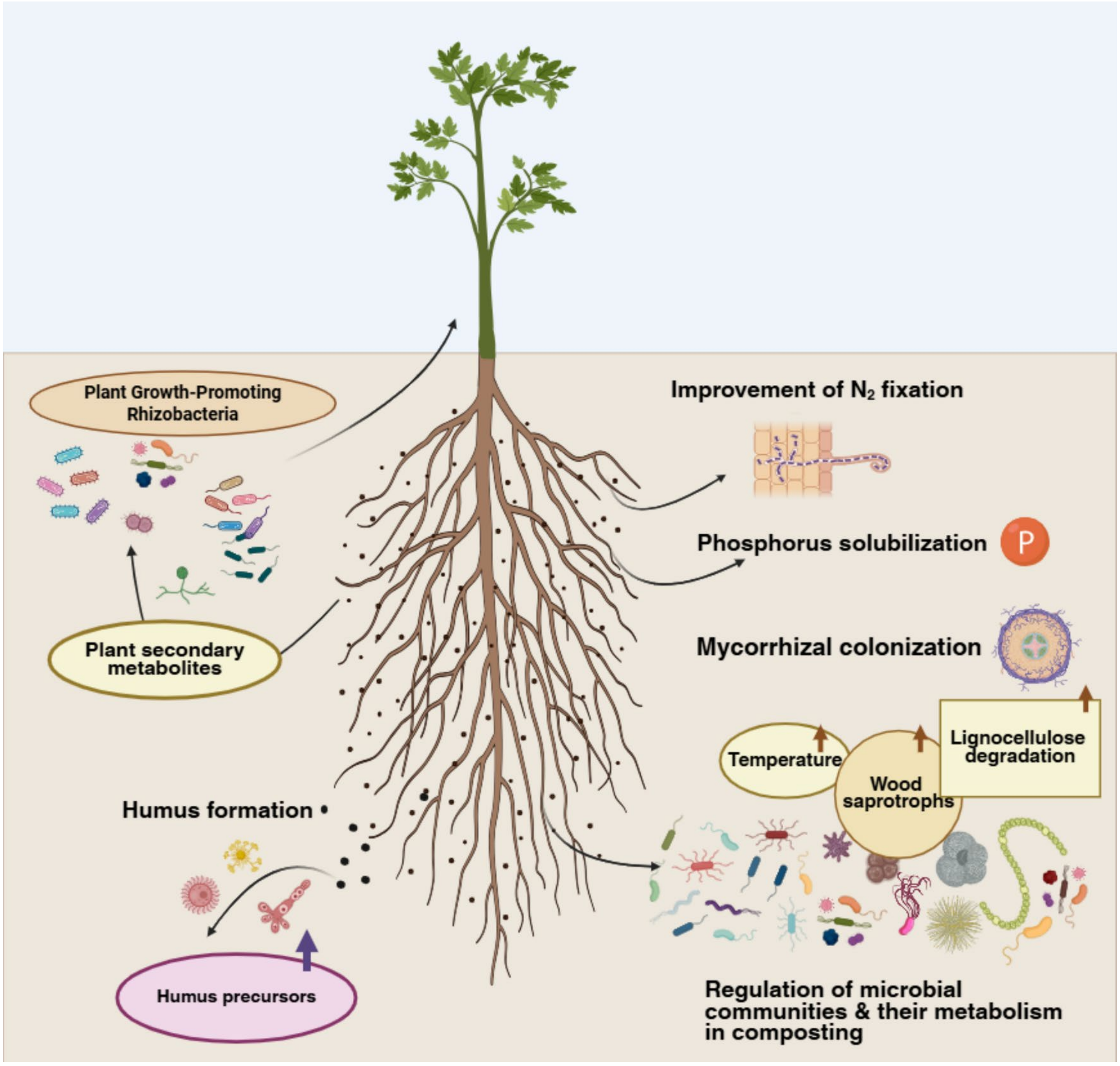

Fig. 6 Plant–microbe interactions leading to enhanced soil fertility and health upon biochar addition

Table 3 Biochar-Induced Metabolomic Shifts in the Rhizosphere: Insights from Recent Studies

Biochar type	Biochar formulation	Metabolome quantification method	Databases used for metabolite identification	Enriched metabolic pathways in the rhizobiome	References
Biochar Combined with Biological Agents					
Bamboo **800 °C**	2% (w/w) biochar	GC-TOF–MS	LECO-Fiehn Rtx5, FiehnLib	Terephthalic acid, protocatechuic acid, gentisic acid (↑); 9-phenanthrol (↓)	(Lu et al. 2024)
	2% (w/w) biochar + *Pseudomonas putida*			Terephthalic acid, protocatechuic acid, pyrocatechol (↑)	
Wheat straw	Arbuscular mycorrhizal fungi (*Diversispora versiformis*)	LC–MS-MS	HMDB, KEGG	Lipid metabolism in *Phragmites* root exudates; glycerophosphate metabolism (↑)	(Li et al. 2022)
Biochar Under Environmental Stress					
Cornstalk **500 °C**	Uranium-stressed soil with canola planting with 3% biochar	UHPLC-MS/MS	KEGG	Lipid metabolism (e.g., choline, glycerophospholipid, glycerolipid), cutin/suberin/wax biosynthesis (↑); Nucleotide metabolism (ABC transporters, purine) (↑); Amino acid and carbohydrate metabolism (↑)	(Li et al. 2024)
Maize straw **500 °C**	PAH-contaminated soil + 1% biochar	GC-TOF–MS	MetaboAnalyst, NCBI, and PubChem	Glycerophospholipid/glycerolipid metabolism (biochar); Amino acid metabolism (e.g., glycine, serine, threonine, valine, leucine, isoleucine), glutathione, lipid metabolism (steroids, fatty acids) (↑)	(Li et al. 2020, 2019)
Cotton straw **450 °C**	Cd-stressed soil + 3% biochar	UHPLC-MS/MS	KEGG	L-histidine in root exudate correlated to boll number; L-isoleucine, L-arginine, L-histidine ↑ by 11.74%, 47.58%, 33.92%	(Zhu et al. 2023b)
Biochar with Fertilizer or Urea Amendments					
Maize straw **500–600 °C**	Compost (20% mulberry, 80% pig manure); 30 kg pile^{-1}	LC-QTOF	KEGG, National Institute of Standards and Technology	Xenobiotics (bisphenol, quinone), amino acid metabolism (tryptophan, tyrosine), quinolines, phenolics, aromatic amino acids (↑)	(Liu et al. 2023a)
Maize straw **400 °C**	N fertilizer + 2% biochar	LC–MS	HMDB, Metlin, KEGG	Lipid metabolism, xenobiotic degradation, amino acid metabolism, organic oxygen metabolites (↑)	(Xia et al. 2023)
Corn straw **550 °C**	1% biochar + 1% urea + ryegrass	GC-TOF–MS	LECO-Fiehn Rtx5	Phenolic acids (caffeic, shikimic) in roots/soil (↑); Naphthalene/anthracene degradation, pyruvate, butanoate metabolism (↑)	(Liu et al. 2023b)
Sugarcane straw **550–650 °C**	20 t ha^{-1} biochar + 375 kg fertilizer	LC–MS-MS	In-house MS2 database	Pelargonic acid linked to sugar/stalk/height; Pentanelactone (↑ sugar), Berberine (↑ height)	(Fallah et al. 2023)

(↑) Increased, (↓) Decreased, *NI* not indicated, *GC−TOF−MS* gas chromatography coupled with a time−of−flight mass spectrometer, *HMDB* human metabolome database, *KEGG* Kyoto Encyclopedia of Genes and Genomes, *LC–MS* liquid chromatography−mass spectrometry, *LC–MS−MS* liquid chromatography with tandem mass spectrometry, *LC−QTOF* liquid chromatography quadrupole time−of−flight, *UHPLC−MS/MS* ultra−performance liquid chromatography−tandem mass spectrometry and function of the rhizobiome (Hildebrand et al. 2023). For example, a study characterizing the rhizosphere metabolomes of three plant species — *Piper auritum, Hibiscus rosa sinensis* and *Clitoria fairchildiana* using solution-state NMR and high-resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS) revealed species-specific stress responses to drought, with notable shifts in the composition of primary metabolites (Hildebrand et al. 2023). The metabolic profiles also indicated a shift toward soluble sugars in root exudates in response to stress. Moreover, combining rhizosphere metabolomics with 16S rRNA gene amplicon sequencing of the rhizobiome revealed that bacterial communities differed in a plant species-specific manner, irrespective of drought conditions (Hildebrand et al. 2023). Hence, the rhizosphere metabolome was more affected by the root exudates than the rhizobiome. This integration enabled a comprehensive understanding of the factors influencing a plant's stress response, highlighting the complementary nature of metametabolomics and metagenomics analyses. Without this integration, a metagenomics approach alone would have provided an incomplete picture of the complex interactions within the rhizosphere.

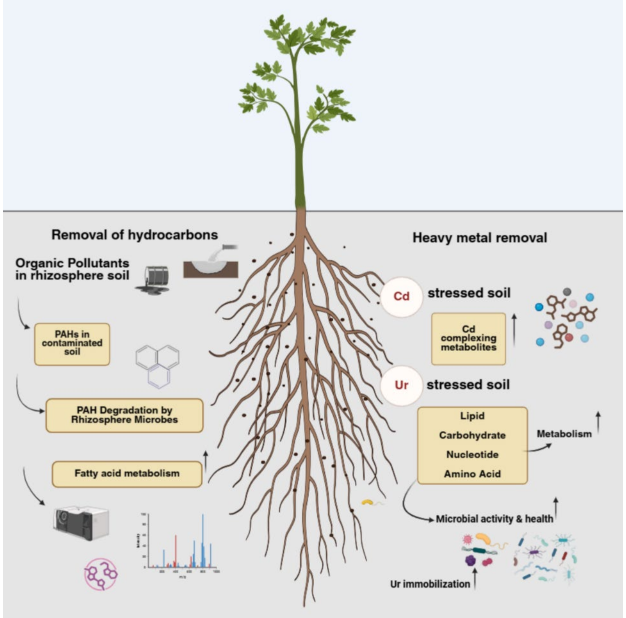

Fig. 7 Plant–microbe interactions leading to soil remediation upon biochar addition

6.2 Impact of biochar on rhizobiome metabolism

Understanding the impact of biochar on rhizobiome metabolism is crucial, given the increasing use of biochar in soil amendment. A comparative metametabolomics approach proves valuable for investigating the differential recruitment of the rhizobiome following biochar addition. Moreover, coupling metabolomics with metatranscriptomics can identify DEGs and differentially accumulated metabolites (DAMs), providing insights into upregulated or downregulated pathways.

For instance, a metabolomic profiling study examining the molecular response of *Quijiao No.* 1 pepper to *Fusarium oxysporum* f sp. *capsici* infestation under bamboo biochar application revealed the accumulation of over 700 metabolites in pepper fruit and leaf samples (Zhu et al. 2021). Among these metabolites, DAMs involved in phenylpropanoid/flavonoid biosynthesis were detected under biochar amendment, suggesting the induction of plant immune response pathways (Zhu et al. 2021). Since many metabolites may accumulate in plants due to the *Fusarium* wilt alone, comparing the metabolic profiles with or without biochar addition is crucial. Similarly, biochar has also been implicated in petroleum hydrocarbon biodegradation, increase of plant biomass and enhancement of soil microbiota (Liu et al. 2023a).

Most studies incorporating metabolomics have focused on the potential of biochar in soil remediation, as depicted in Fig. 7 and Table 3. For example, a metabolomics study reported the reduction of cadmium (Cd) availability in soil by cotton (*Gossypium hirsutum* L.) straw-derived biochar (3% w/w), highlighting the differential upregulation of metabolites involved in soil Cd complexation (Zhu et al. 2023b). While spectroscopic analysis can determine the potential of biochar in reducing soil Cd content, a comparative metametabolomics approach provides a broader understanding of the underlying mechanisms.

In the context of phytoremediation, gas chromatography analysis of the ryegrass (*Lolium perenne* L.) rhizosphere metabolome following the co-application of 1% urea and 1% biochar (derived from corn straw pyrolyzed at 550 °C) revealed the enrichment of 18 metabolites and the upregulation of naphthalene and anthracene degradation, pyruvate metabolism and butanoate metabolic pathways associated with organic pollutant degradation pathways (Liu et al. 2023a). Moreover, the comparative metametabolomics analysis showed that urea had a greater impact on soil metabolites than biochar, informing decisions on optimal soil amendments for bioremediation (Liu et al. 2023a).

However, there remains a knowledge gap regarding the interactions between biochar, soil physicochemical properties, biotic (pathogens and pests) and abiotic (harsh environmental conditions) stresses, plant species, and species-specific microbial recruitment on the rhizosphere metabolome that shape rhizobiome communities. Comprehensive studies are needed to unravel these complex interactions and their impact on the rhizosphere metabolome under biochar amendment. Such studies will be instrumental in optimizing biochar application for sustainable soil management and ecosystem health.

6.3 Holistic view of the effect of biochar through the lens of metaomics technologies

The simultaneous application of metagenomics, metatranscriptomics, metaproteomics, and metametabolomics offers a comprehensive approach to unravelling the underlying molecular mechanisms and obtaining a holistic view of the overall effects of biochar on improving the soil environment and enhancing plant growth and development. By integrating multi-omics technologies, researchers can better understand the molecular, physiological, metabolic, and biochemical responses of the rhizobiome to biochar amendment. However, as indicated in Table 4 and 5, most existing literature has not incorporated more than two metaomics technologies in a single study despite the potential benefits. Several factors contribute to this limitation, including soil heterogeneity, challenges in computational methods for bias exclusion and data analysis, the need for extensive efforts, high instrumentation costs, and potential methodological pitfalls (Peng et al. 2024). Therefore, improvements in methodologies and instruments are necessary to advance research aimed at integrating multi-omics approaches for studying the effects of biochar.

7 Challenges, limitations, and future perspectives

Despite their many advantages, using metaomics approaches to understand the effect of biochar on the rhizobiome faces several challenges and limitations. The complexity of the rhizosphere, with its diverse biotic and abiotic components, poses a significant challenge to obtaining representative samples, as multiple variables and parameters must be considered (Priya et al. 2021).

The interplay of various factors complicates metagenomic studies. Biochar alters soil physicochemical properties, which can be subsequently influenced by environmental conditions. Hence, the same biochar type can have differing effects on the same soil type at different temperatures. In addition, biochar addition alters root exudation, impacting the rhizobiome metagenome as environmental conditions and nutrient availability affect microbial populations. Moreover, the choice of extraction method and lysis technique can introduce biases and affect the accuracy of metagenomic data (White III et al., 2017). Much of the challenges revolving around metagenomics studies can be eliminated by the introduction of standardized protocols for the collection of soil samples and extraction of DNA. Adherence to a standard protocol would also facilitate consistent and reliable results, enabling fair comparison. This would also require accurate measurements of soil physicochemical properties such as pH, SEC and temperature before and after biochar amendment to gain a reliable picture of microbial community dynamics.

Metatranscriptomics studies face challenges such as the short half-life and low yield of mRNA (Peng et al. 2024), the risk of plant RNA contamination (Bashiardes et al. 2016), interference from humic acids (Ijaz et al. 2021), and the influence of host plant genetics on the rhizobiome that complicate the identification of genes impacted explicitly by biochar addition. Therefore, metatranscriptomics studies could also benefit from the use of standardized protocols, incorporating buffers with RNAase inhibitors and metal-chelating ligands such as ethylene diamine tetra acetic acid (EDTA). Thus, the use of Tris–EDTA buffers for storage after collection till the point of analysis would enable a better snapshot of the active microbial community. The lack of interest shown in metatranscriptomics studies could also be due to the inability of metaproteomics and metametabolomics to determine the final products of gene expression. However, despite these challenges, a metatranscriptomics approach cannot be overlooked for identifying functional microbial communities in the rhizobiome and connecting physiological and functional processes in the rhizosphere with biochar addition.

Protein extraction difficulties hinder metaproteomic studies due to high microbial diversity and abundance in the rhizobiome (Priya et al. 2021), while the presence of metabolites from the rhizobiome as well as plants complicates comprehensive metabolic profiling (Ijaz et al. 2021). Furthermore, the lack of appropriate software for multi-omics analyses of the rhizobiome limits the discovery of novel microorganisms, metabolic pathways, and proteins affected by biochar addition (Ijaz et al. 2021). Thus, the development of advanced bioinformatics tools is a dire need for advancing omics studies of this nature. Therefore, the need for collaboration of systems biologists with bioinformaticians, computational biologists and software developers can be understood to accelerate these studies.

Despite these challenges, addressing them can facilitate the formulation of biochar with optimal properties for improving plant growth and soil health. By integrating omics analyses, predictive models can be developed to deduce the response of microbial communities and plants to biochar addition for developing sustainable agricultural practices.

Therefore, overcoming current research gaps and addressing challenges in omics analyses can lead to a better understanding of the role of biochar in the rhizobiome, enabling the development of novel strategies for enhancing plant health and improving soil ecosystem functions, such as nutrient cycling and carbon sequestration, through appropriate biochar application.

Table 4 Biochar Effects on Plant Physiological and Molecular Responses

Plant species	Biochar type	Study target	Plant physiological indices	Omics Used	Key plant responses	
Arachis hypogaea (peanut)	Charred peanut shells (pyrolyzed at 450 ℃)	Impact of biochar in micro-plastic contaminated soil	Root growth improved	Transcriptomics	↑ Antioxidant genes, lignin synthesis, nitrogen transport, energy metabolism	(Yang et al., 2024)
Triticum aestivum (winter wheat)	Apple tree branches (pyrolyzed at 500 ℃)	Salt-alkali stress tolerance	↑ Height by 17.33%	Metabolomics	↑ Metabolites in stress-related pathways (pyrimidine, valine, etc.), ↑ Antioxidants	(Duan et al. 2023)
Radix pseudostellariae	Rice hull (pyrolyzed at 500 ℃)	Alleviating replanting disease	↑ Leaf area and biomass	NI	NI	(Wu et al. 2022)
Solanum lycopersicum (tomato)	Greenhouse plant wastes (pyrolyzed at 350 ℃)	Resistance to *Fusarium* crown rot	Plant height, photosynthetic rate; ↓ Disease mortality (57%)	Transcriptomics	↑Defense/growth genes, ↑ Jasmonic acid/auxin pathways, ↓ Salicylic acid signaling	(Jaiswal et al. 2020, 2017)
Trifolium pretense L. (red clover)	Corn straw (pyrolyzed at 500 ℃)	Pb-contaminated soil	↑ Stem length (73.88%) and biomass (197.75%)	NI	NI	(Meng et al. 2023)
Myrica rubra (Dongkui bayberry)	Plant biochar fertilizer (18–24% biochar)	Assess decline disease, vegetative growth, and fruit quality	↑ Branch length (56.65%), ↑ leaf chlorophyll (26.65%), ↑ fruit weight (22.05%)	NI	NI	(Ren et al. 2023)
Capsicum annuum L. (pepper variety *Qujiao No.1)*	Bamboo biochar	Investigate resistance to *Fusarium* wilt	↑ Enzymes for resistance, ↑ physiological performance, ↓ disease incidence	Transcriptomics & Metabolomics	↑ Protein processing (fruit), ↑ plant–pathogen interaction, ↑ photosynthesis, ↑ phenylpropanoid biosynthesis	(Zhu et al. 2021)
Chinese cherry ‘Manaohong’ (*Cerasus pseudocerasus* Lindl.)	Tobacco straw biochar (pyrolyzed at 400 ℃)	Investigate effects on growth and nutrient uptake	↑ Growth, photosynthesis, and uptake of N, P, K, Mn	Transcriptomics	↑ Indoleacetic acid signaling, ↓ Abscisic acid transduction	(Yang et al. 2022)

NI = Not Indicated

Table 5 Biochar Effects on Rhizomicrobiome via Metagenomics

Plant species	Rhizosphere response (via Metagenomics)	Functional shifts	References
Arachis hypogaea (peanut)	↑ Actinobacteriota, Methylomirabilota, Patescibacteria; ↓ Cyanobacteria, Acidobacteriota	Shift in dominant phyla	(Yang et al. 2024)
Triticum aestivum (winter wheat)	↑ *Streptococcus*, *Mycothermus*; ↑ functional genes for motility and signaling	↑ Resilience in saline soil	(Duan et al. 2023)
Radix pseudostellariae	↑ *Pseudomonas*, *Lysobacter*, *Gemmatimonadetes*, *Nitrospira*	↓ *Fusarium*, ↑ fatty acids, gibberellin A53	(Wu et al. 2022)
Solanum lycopersicum (tomato)	↑ *Proteobacteria*, *Firmicutes*	↑ Plant-beneficial taxa	(Jaiswal et al. 2020, 2017)
Trifolium pretense L. (red clover)	↑ *Chitinophaga*, *Sphingomonas*, *Devosia*, *Pseudomonas*	↑ Rhizobacterial restoration in Pb soils	(Meng et al. 2023)
Myrica rubra (Dongkui bayberry)	↑ *Mycobacterium*, *Fusarium*, ↓ *Acidothermus*, *Byssobacter*	↑ Aconitic acid, ↓ palatinol, ↑ pathways incl. ATP-binding cassette transporters	(Ren et al. 2023)
Chinese cherry 'Manaohong' (*Cerasus pseudocerasus* Lindl.)	↓ *Proteobacteria*, *Planctomycetes*, *Nitrospirae*; ↑ *Acidobacteria*, *Gemmatimonadetes*, *Verrucomicrobia*	↑ Glycosyl transferases, carbohydrate-binding modules, glycoside hydrolases, carbohydrate esterases	(Yang et al. 2022)

NI not indicated, (↑) increased, (↓) decreased, *DEGs* differentially expressed genes, *DEMs* differentially expressed metabolites

8 Conclusions

This review shows that biochar is an active agent of microbial selection and functional transformation in the rhizosphere, rather than just a passive soil additive. It has an impact on reprogramming plant–microbe-soil relationships at the molecular level in addition to altering soil structure. These impacts are strongly related to changes in soil pH, nutrient availability, water retention, and porosity brought about by biochar. These changes alter root exudation patterns and selectively enrich microbial taxa with characteristics associated with stress reduction, bioremediation, and nutrient cycling.

Further, this review offers mechanistic insights into how biochar changes the structure, gene expression, and metabolic activity of microbial communities, based on new multi-omics evidence, especially from metagenomics and metatranscriptomics. These functional changes are usually related to certain biological reactions including enzyme synthesis, metabolite exchange, and signalling pathways at the root–soil interface. In particular, key findings have highlighted the use of metagenomics in unveiling how differences in source of biomass and pyrolysis temperature can impact the rhizobiome composition differently, where beneficial microbes have been favoured and pathogenic microbes have been suppressed upon biochar treatment. Studies have also implicated contrasting responses in certain pathways due to the changes in biochar formulation and concentration. These findings present biochar as a dynamic, context responsive mediator of rhizosphere processes whose results differ depending on the type of feedstock, pyrolysis settings, soil properties, and host plant species. Interestingly, these insights also underscore the need for further research on mechanism of biochar action, while equally emphasizing the need for standardized protocols for biochar production in order to explain discrepancies and to facilitate streamlined analyses. The creation of focused, microbiome-informed agricultural methods is made possible by a better mechanistic knowledge of how biochar alters microbial functioning.

Metatranscriptomics approach towards deciphering the effect of biochar has been crucial in unravelling the differential expressions of genes in metabolic pathways responsible for plant growth promotion. These studies could be further supported by metaproteomic analyses to illuminate soil nutrient shifts upon biochar amendment. However, this remains a largely unexplored area due to the lack of expertise and necessary instrumentation for streamlined metaproteomic analyses. A broad picture of the impact of biochar on rhizobiome metabolism requires the use of metametabolomics approaches. Metabolomic profiling of biochar-amended soils has revealed the potential of biochar in plant growth, plant immune responses, pathogen suppression, hydrocarbon biodegradation, as well as soil remediation through organic pollutant and heavy metal removal. Nevertheless, a holistic view of the effects of biochar is only possible through the integration of these multi-omics approaches, which has been greatly hindered at present due to deficiencies in methodologies, expertise and instrumentation.

Applications of precision biochar that are adapted to the soil and crop environment have the potential to increase plant resilience, decrease reliance on chemical inputs, and improve nutrient usage efficiency. Thus, standardising biochar production techniques and analytical procedures across omics platforms must be a top priority for future research in order to fully realise these advantages. The advancement of biochar from a viable soil addition to a strategic tool for designing advantageous plant–microbe interactions ultimately depends on adopting integrated, systems-level techniques. In addition to increasing agricultural output and soil health, such initiatives will significantly support sustainable land management in the face of environmental issues around the world.

Acknowledgements Financial assistance by the RG/2021/AG/02, National Science Foundation, Sri Lanka.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions. National Science Foundation of Sri Lanka, RG/2021/AG/02, Meththika Vithanage.

Data Availability No new data were generated or analysed in this study. Data sharing is not applicable.

Declarations

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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