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Mitigation of Legacy Effects in Invaded Soil via Alien Plant-Derived Biochar

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Keywords: alien invasive plant | biochar | soil fungi | soil legacy effect

ABSTRACT

The introduction of nonnative plant species often leads to extensive land colonization and poses a threat to already limited arable land resources. Restoring invaded land and improving soil fertility are therefore essential. In this study, both biochar derived from the invasive *Chromolaena odorata* and a fungicide were used to improve the soil invaded by this species. The results revealed that fungicide application increased the total biomass of the subsequent plants growing in the invaded soil and significantly altered the recruitment of mycorrhizal and saprophytic fungi. The addition of biochar to invaded soil had a negative legacy effect on subsequent invasive plants but a positive effect on native plants. The combined application of biochar and fungicide effectively diminished the competitive advantage of invasive species, whereas biochar alone markedly increased the competitiveness of native plants. This study proposes a remediation method for mitigating the legacy effects of alien plant invasion that not only suppresses the spread of invasive species but also improves soil fertility and provides potential economic benefits.

1 | Introduction

As global economic integration accelerates and human interactions increase, the invasion of alien species has become more severe. After their introduction, alien plants can alter soil properties to promote their successful establishment. Even after their removal, the modified soil environment can continue to influence the growth of subsequent plants—a phenomenon known as the soil legacy effect (Jing et al. 2022). Soil legacies caused by alien plant invasion pose serious ecological risks and serve as important mechanisms for understanding the success of these invasions. Common removal methods, such as widespread herbicide application and burning, are counterproductive; they impair the soil's vital role as a carbon store and simultaneously increase carbon emissions. Therefore, environmentally friendly strategies are urgently required to remove invasive plants and to eliminate the residual soil legacy effects following their removal.

Upon invading new habitats, alien plants often establish associations with beneficial soil microorganisms, including mycorrhizal fungi and phosphate-solubilizing bacteria, to improve

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Summary

Invasive plants often spread rapidly and degrade soil, making it harder for native species to grow. This study tested a new way to restore soils invaded by *Chromolaena odorata*, a highly aggressive alien plant. We used biochar made from the invader itself and a fungicide to improve soil conditions and support native plant recovery. The results showed that the fungicide boosted plant growth by changing the balance of soil fungi, while biochar suppressed future invasion and promoted native species. When applied together, biochar and fungicide reduced the competitive advantage of invasive plants and improved soil fertility. This eco-friendly approach helps control invasive species, enhances soil health, and can also contribute to more sustainable land use and agricultural productivity.

Practitioner Points

- Biochar derived from invasive plants can reduce their regrowth and promote native vegetation recovery.
- Fungicide improves plant biomass by shifting soil fungal communities toward more beneficial groups.
- Combining biochar and fungicide provides an effective strategy for restoring invaded soils and improving soil fertility.

their mineral nutrient acquisition and confer a competitive advantage. For instance, compared with the two native plants (*Polygonum chinense* and *Paederia scandens*), the invasive *Mikania micrantha* significantly increases the availability of phosphorus in its rhizosphere by promoting the accumulation of phosphate-solubilizing bacteria (*Pseudomonas* and *Enterobacter*), thereby facilitating its invasion (Yin et al. 2020). Alien plants can also rapidly accumulate native soil pathogens in the invaded area that have a significant inhibitory effect on native plants but little or no impact on themselves, affecting competition between invasive and native plants (Eppinga et al. 2006). For example, the invasive plant *Chromolaena odorata* increases the abundance of the pathogenic fungus *Fusarium semitectum* in the invaded soils, which severely inhibits the growth of two native plants but has no apparent effect on *C. odorata*, thereby promoting its own spread (Mangla et al. 2008). These plant–soil microorganism interactions are key to invasion success.

The microbial changes induced by alien plants can continue to influence subsequent plant growth through legacy effects. Interactions between plants and rhizosphere microorganisms may even lead to heritable changes in plant traits (Jacquiod et al. 2022). Research has shown that fungi persisting in the soil after extreme droughts can influence root architecture and biomass via negative feedback between plants and the soil (Lozano et al. 2022; Shi et al. 2021). The structure of microbial communities in the rhizosphere is closely related to the input of organic carbon from plants (via rhizodeposits and litter) and to soil properties, which in turn can influence plant phenology and alter the distribution of photosynthetic carbon in different plant components (Te Beest et al. 2009; Van Nuland et al. 2021). When *Lasthenia californica* was grown for 3 months in soil invaded by an alien plant (*Aegilops triuncialis*), its flowering was delayed, its aboveground biomass was significantly reduced, and the composition of the soil microbial community was significantly altered (Batten et al. 2008). Such findings show that invasive alien plants can act as pathogen reservoirs and create persistent microbial legacies that affect the regeneration of native vegetation. Hence, urgent action is needed to prevent the spread of invasive plants and the transmission of pathogenic microorganisms that hinder the establishment of local plant communities.

By modifying these microbial legacies in the soil, subsequent plant communities can recruit new microbial assemblages and reestablish a positive plant–soil feedback, thus aiding ecosystem recovery. Research has shown that applying fungicides to invaded soils can disrupt existing pathogen networks, allowing subsequent plants to recruit beneficial microbial networks and restore soil biological activity (Domínguez-Begines et al. 2021; Maron et al. 2013). However, the introduction of soil fungicides can temporarily alter nutrient cycling involving soil microorganisms during the early stages of plant establishment (Schnurr et al. 2025), so nutrient supplements are needed to prevent nutrient deficiencies (Wang and Tang 2024), and measures must also be taken to prevent pollution from harmful fungicide runoff. Biochar has a wide range of applications in soil remediation, including adsorbing organic and inorganic pollutants and binding harmful chemical substances (Cao and Harris 2010; Harindintwali et al. 2023). In addition, the ash content of biochar facilitates plant nutrient uptake. The combination of soil fungicide and biochar can suppress pathogenic microorganisms (Mazhar et al. 2024) while providing the nutrients essential for early plant growth. The effectiveness of this dual approach can be evaluated through plant–soil interactions.

Chromolaena odorata is native to the Americas but invaded tropical regions in Asia and Africa in the mid-19th century. It poses a serious threat to native biodiversity and ecological stability due to its prolific seed bank and rapid spread. In tropical regions of China, with abundant warmth and rainfall, *C. odorata* can quickly accumulate large amounts of biomass and high seed yields. Research has shown that processing alien plants into biochar can effectively inactivate their seeds and rhizomes, thereby controlling their risk of spread to some extent (Feng et al. 2021). We hypothesize that (1) the introduction of alien plants strongly affects soil properties, which significantly affects the subsequent growth of the plants; (2) these effects can be suppressed by the application of fungicide; and (3) the impairment of plant growth caused by fungicides can be alleviated by the addition of biochar, which has a positive effect on subsequent plant growth. To test these hypotheses, we collected soil severely invaded by *C. odorata* in the field, eliminated the influence of the original pathogen community using fungicides, and prepared *C. odorata*-derived biochar through high-temperature pyrolysis. This application of biochar effectively promotes early plant establishment (Xu et al. 2024), helping to achieve soil remediation. This method not only curbs reinvasion but also improves soil fertility and enhances plant productivity, ultimately promoting the stability of the entire ecosystem.

2 | Materials and Methods

Soil samples were collected from areas heavily invaded by the invasive plant *C. odorata*, while uninvaded soil samples served as

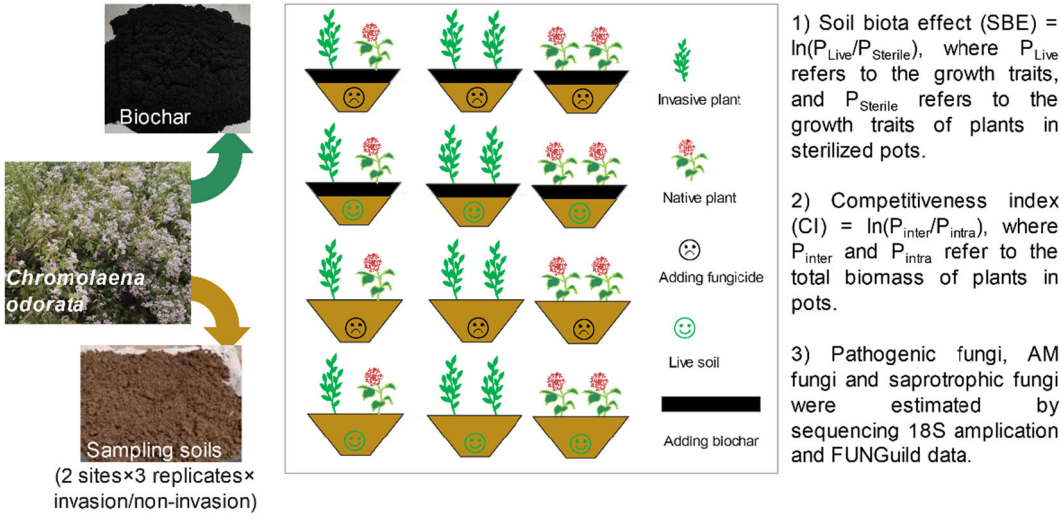

FIGURE 1 | Schematic illustration of the experimental layout.

controls. Surface soil (0–20 cm) was collected and sterilized for use in pot experiments. Biochar utilized in the experiment was produced by anaerobic dry distillation of *C. odorata* biomass in a carbonization furnace. The experimental setup is illustrated in Figure 1, and the specific procedures are detailed below.

2.1 | Preparation of Biochar From the Invasive Plant *Chromolaena odorata*

Biomass of *C. odorata* was collected from invaded sites, air-dried under sunlight, and converted into biochar via anaerobic dry distillation in a carbonization furnace. First, the furnace was purged with pure nitrogen gas to create an anaerobic environment before heating. The temperature was then increased from room temperature to 500°C at a rate of 3°C/min and maintained for 2 h. The resulting biochar was gently removed from the furnace, sieved through a 60-mesh screen, and prepared for use in the pot experiments. The pH of the biochar was determined to be 10.17.

2.2 | Soil Sampling and Fumigation

Soil was excavated from two representative invasion sites: Menglun Town, Mengla County (21°91′ N, 101°26′ E), and Shiliangzi, Xiangming Township, Mengla County (22°12′ N, 101°16′ E). Samples were collected from three replicate plots, spaced 20–50 m apart. An adjacent area of non-invaded soil was used as a control. Surface soil (0–20 cm) was excavated, passed through a 5 cm sieve, and fumigated with Dazomet fungicide. Soil nutrient parameters, including soil organic carbon, total nitrogen, and total phosphorus, are presented in Table S1.

2.3 | Determination of Soil Nutrients and Biochar pH Determination

Soil chemical properties were determined following standard procedures described by Lu (1999). Soil organic carbon (C) was analyzed using the $K_2Cr_2O_7$–H_2SO_4 oxidation method, total nitrogen (N) was measured as Kjeldahl-N, and total phosphorus (P) was measured after HF–$HClO_4$ digestion using the molybdenum blue method. Biochar pH was determined using a pH meter (FE30, Mettler-Toledo, Switzerland) in a biochar-to-water suspension (1:20, w/v) following Nguyen and Lehmann (2009).

2.4 | Pot Experiment

In each five-gallon plastic pot (27 cm diameter, 31 cm height), intraspecific competition occurred among plants of the same species, as well as interspecific competition between *C. odorata* and the native *Triumfetta rhomboidea*. The total number of pots was calculated as: 2 (invaded areas) × 2 (invaded soils) × 3 (soil repetitions) × 3 (species combinations) × 2 (biochar) × 2 (fungicide) = 144. We conducted regular weeding, pest control, and watering to minimize external interference.

The growth period for the potted plants spanned 5 months, from May 20th to October 20th, 2022. (1) Measurement of plant height: At mid-growth, the height of each plant was measured using a tape measure. (2) Measurement of specific leaf area: At mid- to late-growth, five complete leaves were sampled from each pot, and the total leaf area was measured with a leaf area meter. The leaves were then oven-dried (70°C, 36 h). Plant-specific leaf area = total leaf area/total dry weight. (3) Biomass determination: At late-growth, plants were harvested and separated into leaves, stems, and roots. The dry biomass of each component was measured, and total biomass per pot was calculated, along with the proportional contribution of the different components.

2.5 | Illumina Sequencing Analysis of 18S Gene Amplicons

Genomic DNA was extracted from 0.5 g of fresh soil using a FastDNA SPIN kit (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's protocol. The universal primers SSU0817F (5'-TTAGCATGGAATAATRRAATAGGA-3') and 1196R (5'-TCTGGACCTGGTGAGTTTCC-3') were used to amplify

the 18S genes. The amplicons were subjected to paired-end sequencing on an Illumina MiSeq platform following standard protocols at Shanghai Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Paired-end reads obtained from the original DNA fragments were merged using FLASH (Magoč and Salzberg 2011) and subsequently assigned to individual samples using unique barcodes. The sequences were analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) software package (Caporaso et al. 2010), with in-house Perl scripts were employed to analyze beta (between-sample) diversity. Quality filtering of the reads was performed using QIIME's quality filters. Operational taxonomic units (OTUs) were then clustered using the pick_de_novo_otus.py pipeline, and an OTU table was generated. Sequences exhibiting ≥ 97% similarity were assigned to the corresponding OTU. Representative sequences were selected for each OTU, and the taxonomic information for eukaryotic microbes was annotated using a subset of the SILVA 119 database (http://www.arb-silva.de/download/archive/qiime/). The sequencing data were categorized into three functional groups, namely, pathogenic fungi, arbuscular mycorrhizal (AM) fungi, and saprotrophic fungi, based on the data available from FUNGuild (Nguyen et al. 2016). All sequencing data for the fungal genomes have been deposited in the NCBI Sequence Read Archive (http://trace.ncbi.nlm.nih.gov/Traces/sra/) under the accession number PRJNA1002838.

2.6 | Differential Abundance Analysis of the Eukaryotic Microbial Community

Bray–Curtis dissimilarity indices were computed in QIIME to evaluate differences in eukaryotic microbial community composition across treatments. The resulting distance matrices were visualized using nonmetric multidimensional scaling ordination to reveal patterns of community separation. To test for statistically significant differences in beta diversity among treatments, permutational multivariate analysis of variance was performed based on the Bray–Curtis distance matrices.

To evaluate the effects of soil sterilization and biochar addition on the eukaryotic microbial community, two independent differential abundance analyses were performed: (1) Sterilized treatment versus non-sterilized control, and (2) biochar-amended treatment versus non-amended control. Analyses were conducted using the OTU table as input. Differentially abundant taxa were identified using the "DESeq. 2" R package, which models count data with a negative binomial distribution suitable for handling the over-dispersion typically observed in amplicon sequencing datasets. In each comparison, the untreated control group was used as the reference condition. For each OTU, fold changes in relative abundance and associated statistical significance were estimated, with Wald tests applied to assess differences between groups. The resulting *p*-values were adjusted for multiple testing using the Benjamini–Hochberg procedure to control the false discovery rate (FDR). Results were visualized as volcano plots, with log2-transformed fold change (log2FC) on the x-axis, and the negative log10-transformed FDR-adjusted *p*-value (−log10[FDR]) on the y-axis, illustrating both effect size and statistical significance of each taxon. The following thresholds were applied to define significantly altered taxa: Magnitude threshold: $|\log2FC| > 1$ (i.e., more than a two-fold difference in abundance). Significance threshold: $FDR < 0.05$. Based on these criteria, OTUs were classified into three categories: Significantly enriched: $\log2FC > 1$ and $FDR < 0.05$. Significantly depleted: $\log2FC < -1$ and $FDR < 0.05$. Nonsignificant: OTUs not meeting both thresholds. In the final volcano plots, enriched OTUs were highlighted in red, depleted OTUs in blue, and nonsignificant OTUs in gray.

2.7 | Data Analysis

The soil biota effect (SBE) was calculated as $SBE = \ln(P_{Live}/P_{Sterile})$, where P_{Live} refers to the total biomass of plants in pots without fumigation, and $P_{Sterile}$ refers to the growth traits of plants in fumigated soils. $SBE > 0$ indicates a positive soil biotic effect, while $SBE < 0$ indicates a negative effect. The competitiveness index (CI) of plants was calculated as $CI = \ln(P_{inter}/P_{intra})$, where P_{inter} and P_{intra} represent the total biomass of plants in pots under interspecific and intraspecific competition, respectively. These formulas are based on the algorithm proposed by Lekberg et al. (2018).

Linear mixed models (LMMs) were used for data analysis, with the response variables being the growth traits of the plants (total biomass, root biomass, root-to-shoot ratio, plant height, and specific leaf area). Fixed factors included invasive plant presence, microbial treatment (sterilization or not), and biochar addition. Soil source (different invasion areas) was treated as a random factor. The effects of soil biota legacies on the growth of invasive and native plants after invasion were analyzed, as were the restorative effects of biochar on the soil biota legacy. Post hoc multiple comparisons of model estimates across variable levels were conducted using the "emmeans" package. LMMs were fitted and evaluated using the "lmerTest" package. All statistical analyses were performed in *R* (version 3.6.2).

3 | Results

3.1 | Fungicide and Biochar Improve the Growth of Subsequent Plants

Soil fumigation significantly increased the total biomass and plant height of subsequent plants grown in invaded soils (Figure 2B and Figure S1B). The addition of soil fungicide resulted in a marked reduction in the root-to-shoot ratio of native plants (Figure 2F) and a concurrent decrease in the specific leaf area of invasive plants (Figure S1D). In contrast, the addition of biochar resulted in a noteworthy increase in both the root-to-shoot ratio and specific leaf area of indigenous plant species (Figure 2E and Figure S1C).

3.2 | Response of the Eukaryotic Microbial Community to Fungicide and Biochar Addition

Soil fumigation, biochar addition, and their interaction significantly altered the structure of the soil eukaryotic microbial community (Figure 3A). Soil fumigation resulted in the enrichment of 20% of OTUs and the depletion of 13%, whereas biochar amendment affected 0.7% of OTUs (Figure 3C,D). Biochar slightly increased the abundance of pathogenic fungi while modestly reducing that of arbuscular mycorrhizal and saprotrophic fungi (Figure S2A,C,E). In contrast, soil fungicide treatments had a pronounced impact on both mycorrhizal and saprotrophic fungi (Figure S2D,F). These results indicate that biochar and fungicide influence soil fungal

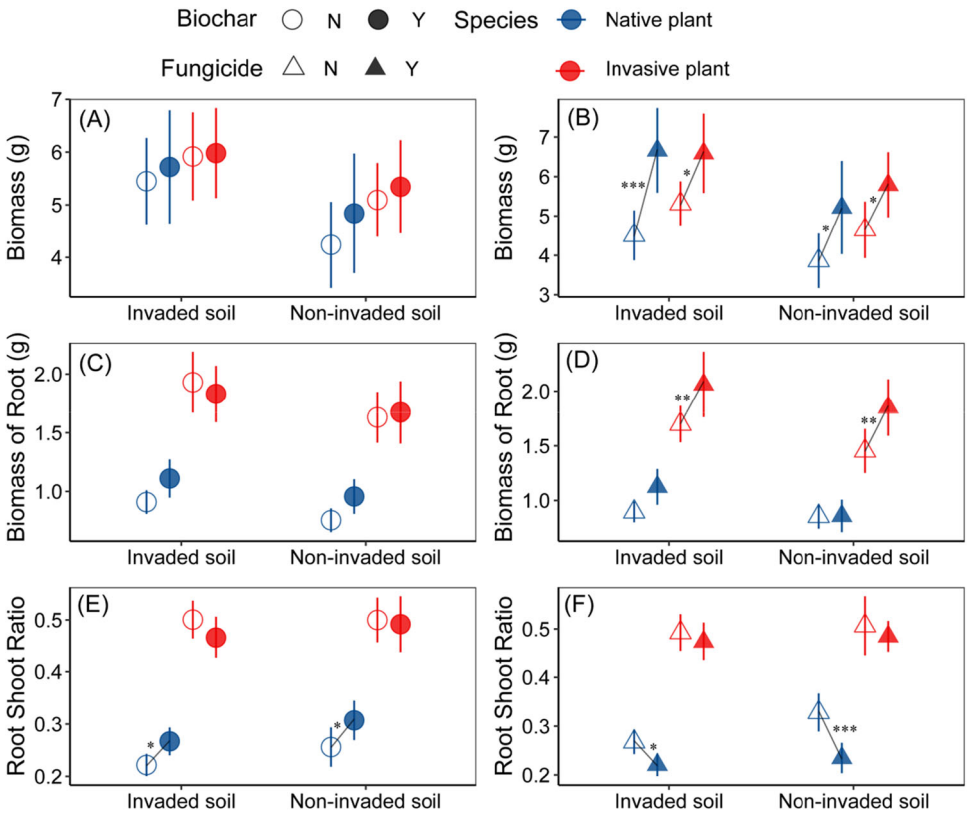

FIGURE 2 | Effects of fungicide treatment and biochar addition on biomass and allocation in subsequent plants. Panels A–B compare the effects of fungicide and biochar addition (Y) versus non-addition (N) on subsequent plant biomass. Panels C, D present root biomass, while panels E, F show the root-to-shoot ratio. Dots represent the mean ± SE. *, **, and *** indicate statistical significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

communities through different mechanisms: Biochar enhances nutrient dynamics but may slightly suppress beneficial fungi, whereas fungicides directly alter the fungal population balance.

3.3 | Mitigation of Biological Legacy Effects Through Biochar

Legacy effects from invasive plant-altered soils hampered biomass accumulation in subsequent invasive plants, resulting in a negative legacy effect (Figure S3A). The application of biochar to invaded soils mitigated these negative residual effects of the soil biota on native plants while increasing the suppressive effect on invasive plants (Figure S3B). Consequently, biochar improved the competitive advantage of native species and reduced that of invasive plants (Figure S4A). Biochar application also strengthened the residual effects of eukaryotic microorganisms, with a significant negative correlation observed between the strength of residual soil effects on root systems and the diversity of soil microorganisms (Figure 4). Furthermore, the utilization of biochar diminished the adverse impacts of microbial legacies on root growth (Figure 5).

4 | Discussion

4.1 | Soil Legacy Effects After Invasion of Alien Plants

The soil biota remaining after alien plant invasion can exert inhibitory effects on the biomass accumulation of subsequent plants. When alien plants enter a new habitat, they often encounter fewer above- and below-ground natural enemies compared with their native range. This reduction in biotic resistance allows invasive plants to exert greater influences on the soil environment, including changes in soil nutrients, enzyme activity, microbial community structure and diversity, and soil fauna (Lau and Lennon 2012). These plant-induced changes create a soil "memory" that continues to influence the growth of subsequent plant communities.

In this study, we examined how the retention of soil biota influences subsequent plant growth by comparing plant performance in fumigated (reduced soil biota) and non-fumigated (intact soil biota) soils. Following suppression of biological activity in invaded soils, subsequent plants accumulated greater biomass and exhibited increased growth, indicating significant negative legacy effects associated with the invaded soil biota. Soil fumigation reduces the feeding of soil organisms on plant roots and the uptake of root secretions by microorganisms, indirectly decreasing the downward flow of photosynthetic carbon and increasing the amount of biomass invested in the formation of aboveground plant components (Li et al. 2023).

4.2 | Eukaryotic Microbial Community Assembly in Response to Fungicide and Biochar

Our findings show that fungicides, biochar, and their interactions significantly altered the composition of the soil eukaryotic microbial community, with fungicides having the more

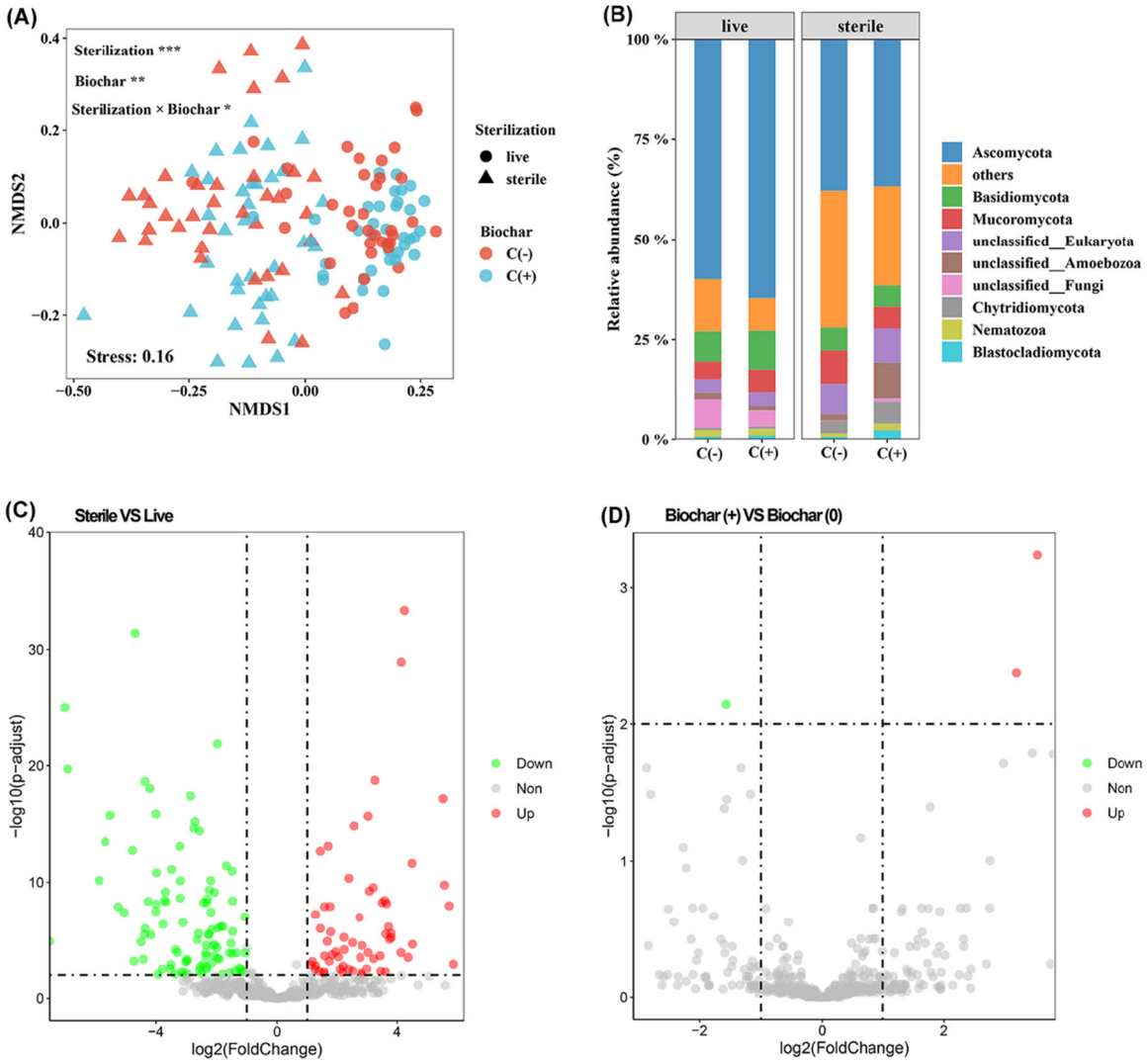

FIGURE 3 | Eukaryotic microbial community responses to biochar and fungicide addition. Panels A,B show the overall eukaryotic microbial community structure. Panels C,D illustrate the degree of changes induced by fungicide and biochar treatments. *, **, and *** indicate statistical significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

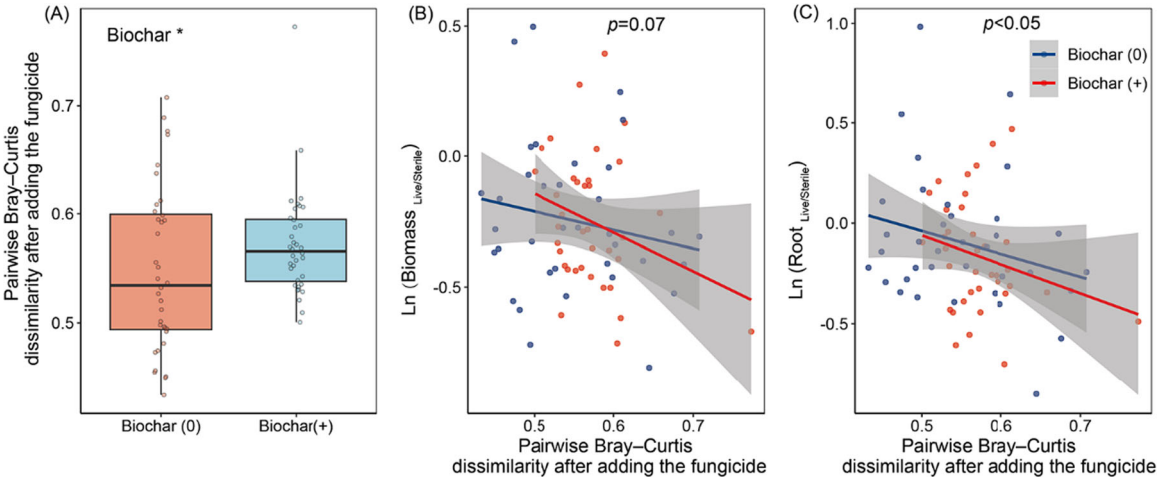

FIGURE 4 | Effects of eukaryotic microbial communities on plant biomass and biomass allocation following biochar addition. Box-and-whisker plots display the median and the 25th and 75th percentiles. The significance of biochar treatment was evaluated using a linear mixed-effects model. *Indicates statistical significance at $p < 0.05$.

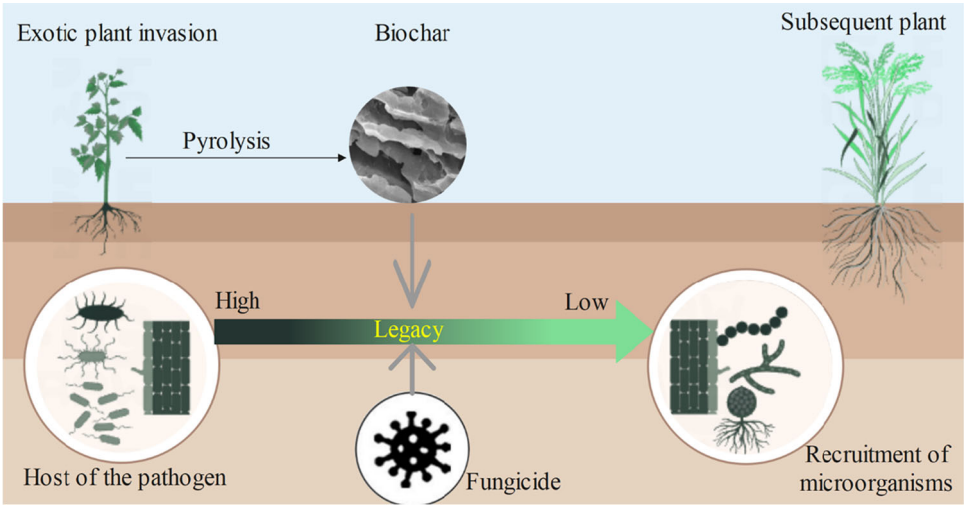

FIGURE 5 | Conceptual illustration showing the remediation of eukaryotic microbial legacy effects through the addition of biochar and fungicide.

pronounced effects (Figure 3). This suggests that the soil microbial legacy is altered after fungicide application, and that subsequent plants may recruit new microbial communities. The similar effects of fungicides on soil biota have also been documented in previous studies (Chen et al. 2022a, 2022b; Eo and Park 2014). We also observed that soil fungicide altered the abundance and balance of functional fungal groups overall (Figure S2). The application of fungicides may simultaneously reduce both pathogenic and beneficial microorganisms in the soil. However, because microorganisms are ubiquitous in the surrounding environment, they can serve as a source pool for the reassembly of the soil microbial community. Plants will soon recruit broad-spectrum mycorrhizal fungi to assist in nutrient and water absorption, as well as saprophytic fungi to decompose organic matter and release nutrients. This accounts for the varying responses of soil functional fungal groups to fungicides.

The structure and function of the plant rhizosphere microbial community are closely related to the inputs of plant-derived organic carbon (rhizosphere sedimentary carbon and litter) and soil physicochemical properties (Zhang et al. 2019). Conversely, soil microorganisms can affect the phenology of aboveground plant leaves and the accumulation of biomass through their effects on nutrient cycling and availability. Although our results indicate that soils invaded by *C. odorata* negatively affected native plant growth—and that fumigation and biochar alleviated these effects—we acknowledge that these results provide correlative rather than causal evidence for pathogen-mediated biotic legacy effects. The fungal community data identified increases in pathogenic functional groups (based on FUNGuild classification), but we were unable to isolate specific pathogenic taxa in pure culture. Future studies incorporating pathogen isolation, culture, and reinfection experiments will be essential to confirm the causal mechanisms following Koch's postulates.

The application of soil fungicides weakens microbial memory and directly affects soil nutrient turnover, which can impair nutrient uptake by subsequent plants. The addition of biochar can enhance soil aggregate stability and water retention, improve nutrient availability, and promote greater microbial community diversity and activity (Hue 2020). Collectively, these improvements in soil properties are likely to support and stimulate plant growth. Biochar can also adsorb certain chemical compounds (such as allelochemicals) present in the soil, thereby reducing their inhibitory effects on plant growth and promoting overall plant performance. The combined use of biochar and fungicides produced clear interactive effects, supporting more stable plant–soil interactions and improving the performance of subsequent plants.

4.3 | The Remedial Effect of Biochar on Soil Legacy Effects

In this study, we emphasize that the introduction of invasive plant species can alter the structure and composition of the soil microbial community, which may subsequently exert legacy ("soil memory") effects that negatively influence the growth of subsequent plant species. Invasive plants can also alter the physicochemical properties of the soil—such as pH, organic matter content, and nutrient availability—which further shape microbial communities and soil biota. Biochar contains ash-derived elements that can directly support plant growth. In addition, its incorporation can improve soil physical structure, enhance permeability, and create a more favorable soil environment, thereby indirectly promoting plant growth (Zhang et al. 2021). Owing to its relatively large specific surface area, biochar can also adsorb allelochemicals in the soil (Alshahrani and Suansa 2020; Chen et al. 2022a, 2022b), which may further contribute to improved plant growth.

Overall, our study highlights the importance of considering the legacy effects of invasive plants on soil environments and subsequent plant growth. The soil "memory" created by invasive plants can significantly affect the growth and survival of subsequent plants, with long-term consequences for ecosystem functioning. It is therefore important to take measures to prevent the introduction and spread of invasive plants while restoring and rehabilitating invaded ecosystems to minimize

their impact on soil environments. Converting invasive plants into biochar can destroy their seeds and rhizomes, thereby helping to control their spread. Processed biochar provides nutrients, adsorbs inhibitory chemicals, and improves soil aggregation, directly promoting plant growth. Additionally, the use of biochar effectively alleviates initial nutrient deficiencies and root damage caused by fungicide application while simultaneously remediating the environmental pollution associated with fungicide use.

Combined biochar and fungicide treatments alleviate soil legacy effects resulting from invasive plant invasion, supporting more effective management strategies and improved land use outcomes. Beyond the system studied here, this approach has the potential to be applied to other invasive species and ecosystems, offering a sustainable and versatile strategy for mitigating invasion impacts, restoring soil health, and enhancing ecosystem resilience across diverse environmental contexts.

While invasive plant-derived biochar shows promise for improving soil legacy effects, several practical limitations should be noted. Scaling up production may be challenging due to biomass availability and processing requirements. Pyrolysis can generate emissions if not carefully managed, and the cost of production and application may limit adoption in some regions. Addressing these constraints will be important for translating these experimental results into practical soil restoration strategies.

5 | Conclusions

We used the invasive species *C. odorata* to produce biochar, which was then combined with a fungicide to alleviate the legacy remaining after invasion. Soil fungicide application significantly modified the abundance of mycorrhizal and saprophytic fungi, influencing the growth of subsequent plants. The incorporation of biochar and fungicide into invaded soils can significantly reduce the competitive ability of nonnative plants while improving the competitiveness of native species. Our findings demonstrate that biochar application effectively mitigates legacy effects in invaded soils and highlight the importance of soil remediation for restoring native plant diversity.

Author Contributions

Weitao Li: conceptualization, validation, writing – original draft, data curation. **Hui Ning:** visualization, data curation. **Yangping Li:** writing – review and editing. **Mingzhu Zhang:** writing – review and editing. **Ruifang Wang:** writing – review and editing. **Yulong Zheng:** visualization, conceptualization, data curation, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Figure S1: The subsequent plant height and specific leaf area under the treatment of fungicide and biochar addition. Panels A-B compare the effects of fungicide and biochar addition (Y) and non-addition (N) on subsequent plant height. Panels C-D focus on specific leaf area. Dots show the mean ± SE. * and *** indicate statistical significance at $p<0.05$ and $p < 0.001$, respectively. **Figure S2:** The response of functional fungi to the addition of biochar and fungicide. Panels A-B compare the effects of fungicide and biochar addition (Y) and non-addition (N) on soil pathogens. Panels C-D focus on AMF, while panels E-F examine the saprotroph. Dots show the mean ± SE. * indicate statistical significance at $p < 0.05$. **Figure S3:** Patterns of soil legacy effects after exotic plant invasion (A) and their alleviation through biochar amendment (B). Dots show the mean ± SE. * indicate statistical significance at $p < 0.05$. **Figure S4:** Effects of biochar (A) and fungicide (B) on competition between invasive and native plants. Dots show the mean ± SE. **Table S1:** The characteristics of soil nutrients from different sampling sites. Data are presented as mean ± SE ($n = 3$). SOC refers to soil organic carbon, TN to total nitrogen, and TP to total phosphorus.