

Mitigation of legacy effects in invaded soil using alien plant-derived biochar

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Research Article

Keywords: Alien invasive plant, biochar, soil legacy effect, soil pathogenic fungi

Posted Date: December 15th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3663026/v1>

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Abstract

Aims

The introduction of non-native plant species often leads to extensive colonization of land and poses a threat to the already limited resources of arable land. Restoring invaded land and improving soil fertility are therefore essential.

Methods

In this study, biochar from invader *Chromolaena odorata* and fungicide were jointly used to improve soil invaded by this species.

Results

The results show that fungicide increase the total biomass of the subsequent plants grown in the invaded soil. The soil fungicide reduces the occurrence of pathogenic fungi in invaded soil and at the same time promotes the recruitment of mycorrhizal fungi and saprophytic fungi by the plants. The addition of biochar to the invaded soil has a negative legacy effect on subsequent invasive plants, while it exerts a positive enhancement effect on native plants. The simultaneous application of biochar and fungicide to soils invaded by alien plants can effectively diminish the competitive advantage of invasive species, while biochar can markedly increase the competitive advantage of native plants.

Conclusions

This study proposes a remediation method for legacy effect after alien plant invasion that not only effectively controls the spread of alien plants, but also improves soil fertility and increases economic benefits.

1. Introduction

As the process of global economic integration accelerates and human interactions increase, the invasion of alien species becomes more serious. After the introduction of alien plants, soil properties are altered to promote successful invasion. Even after removal of the alien plants, the remaining soil environment can continue to influence the growth of subsequent plants, referred to as soil legacy effects (Jing et al. 2022). Soil legacies caused by alien plant invasion pose serious ecological risks and serve as important mechanisms for understanding the successful invasion of alien plants. Currently, there are also many problems with alien plant removal measures, such as large-scale herbicide use and burning, which not only damage the soil's "sink" function but also increase carbon emissions. There is an urgent need to

take environmentally friendly measures to remove alien plants. After the removal of alien plants, the removal of soil legacy effects is also particularly urgent.

On the one hand, when alien plants invade a new habitat, they recruit some beneficial microorganisms, such as mycorrhizal fungi and phosphate-solubilizing bacteria, which help the plants to absorb minerals. It has been found that the invasive plant *Mikania micrantha* significantly increases its content of available phosphorus in the rhizosphere compared to the two native plants *Polygonum chinense* and *Paederia scandens*, and promotes its own invasion through the accumulation of phosphate-solubilising bacteria (*Pseudomonas* and *Enterobacter*) (Yin et al. 2020). On the other hand, alien plants can rapidly accumulate pathogens of native plants in the invaded area. These pathogens often have a significant inhibitory effect on native plants but not on the invasive plants, 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 area it has invaded, which can significantly inhibit the growth of two native plants but has no apparent effect on *C. odorata*, thereby promoting *C. odorata* invasion (Mangla et al. 2008). The interaction between plants and soil microorganisms is an important mechanism for their successful invasion.

The changes in soil microorganisms caused by previous plant stages can influence subsequent plant growth through interactions between plants and soil microbes. The interaction between plants and the rhizosphere microorganisms living with them leads to heritable changes in plant traits (Jacquiod et al. 2022). Research has shown that fungi left in the soil from previous extreme droughts can influence root architecture and biomass through a negative feedback between plant and 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 the plant (rhizodeposits and litter) and soil properties, which in turn can influence plant phenology and alter the distribution of photosynthetic carbon in the different plant components (Te Beest et al. 2009; Van Nuland et al. 2021). After *Lasthenia californica* was planted for three months in soil invaded by alien plants (*Aegilops triuncialis*), the flowering time of *L. californica* was delayed, the aboveground biomass was significantly reduced and the composition of the microbial community in the soil was significantly altered (Batten et al. 2008). Invasive alien plants become hosts for pathogens and create a microbial legacy in the soil that affects the growth of subsequent plants. Urgent action is needed to prevent the spread of invasive plants and the transmission of pathogenic microorganisms that affect the establishment of local plant communities.

By influencing the microbial legacy in the soil, subsequent plant communities can recruit new microbial communities and establish a positive plant-soil feedback, restoring native ecosystems. Research has shown that the application of fungicide to soil can disrupt the existing network of soil pathogens, allowing subsequent plants to recruit new microbial networks and restore soil biological activity (Dominguez-Begines et al. 2021; Maron et al. 2013). The introduction of soil fungicide inevitably affects nutrient cycling involving soil microorganisms in the early stages of plant establishment, so nutrient supplements are needed to compensate for nutrient deficiencies. Measures must also be taken to prevent pollution from harmful fungicide runoff. Biochar has a wide range of applications in soil

remediation, including adsorption of organic and inorganic pollutants in the soil and binding of chemical substances (Cao and Harris 2010; Harindintwali et al. 2023). In addition, the ash content in biochar facilitates nutrient uptake by plants. The combination of soil fungicide and biochar can block the effects of pathogenic microorganisms on later plant growth, while biochar can provide the nutrients needed for early plant growth. The effects of soil remediation by combination of fungicide and biochar can be evaluated through plant-soil interactions.

Chromolaena odorata is native to North, Central and South America. It invaded tropical regions in Asia and Africa in the mid-19th century and poses a serious threat to the growth, biodiversity and ecological security of native plants in the invaded areas due to its large seed bank, rapid spreading ability. In the tropical regions of China with abundant water and heat, *C. odorata* can quickly accumulate a large biomass and high seed yield. Research has shown that preparing alien plants into biochar can effectively inactivate the seeds produced by the plants and their rhizomes, controlling the spread risk of the alien plants 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; 3) the impairment of plant growth caused by fungicide can be alleviated by the addition of biochar, which has a positive effect on subsequent plant growth. We collected soil severely invaded by *C. odorata* in the field, eliminated the influence of the original pathogen community in the soil by fungicide and prepared *C. odorata* into biochar by high temperature pyrolysis. The application of biochar effectively promotes early plant establishment (Xu et al. 2023), achieving the goal of soil remediation. This technology not only controls the spread of alien plants, but also increases the yield of above-ground plants, which benefits the stability of the entire ecosystem.

2. Materials and methods

2.1. Preparing biochar by using invasive plant *Chromolaena odorata*

In the area where *Chromolaena odorata* had invaded, we collected the biomass, dried it in the sunlight and processed it into biochar. We used a carbonization furnace to produce the biochar by the anaerobic dry distillation method with a temperature setting of 3 °C/min heating from room temperature to 500°C and maintained this temperature for 2 hours. The resulting biochar was sieved through a 60 mesh screen and used for the pot experiments.

2.2. Soil sampling and sterilization

Excavate soil from two typical invasive areas: Menglun Town, Mengla County (21°91' N, 101°26'E) and Shiliangzi, Xiangming Township, Mengla County (22°12' N, 101°16'E). Use non-invasive soil as control, and select three sampling points for each sampling area with a distance of 20–50 meters between point

repetitions. Excavate surface soil (0–20 cm), sieve it through a 5 cm sieve, and was fumigated with Dazomet fungicide.

2.3. Pot experiment

In each pot, there was intra-specific competition among plants of the same species, as well as interspecific competition between *Chromolaena odorata*, an invasive species, and *Triumfetta rhomboidea*, a native plant. The total number of pots was calculated as follows: 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 interference from pests, diseases, and weeds.

(1) Measurement of plant height: At mid-growth, the height of each plant was measured with a tape measure. (2) Measurement of specific leaf area: At mid to late plant growth, 5 complete leaves were taken from each pot and the total leaf area of the plant leaves was measured with a leaf area metre. The leaves were then dried (70°C, 36 h). Plant-specific leaf area = total leaf area/total dry weight. (3) Biomass determination: The biomass and leaf, stem and root biomass of each pot were harvested at the later stages of plant growth. The total biomass of each pot was calculated, as well as the proportion of the different components in the biomass.

2.4. Illumina sequencing analysis of 18S gene amplicons

Genomic DNA was extracted from 0.5 g of fresh soil samples using a FastDNA™SPIN kit (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's protocol. The universal primers SSU0817F (5'-TTAGCATGGAATAATRRATAGGA-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 a standard protocol at Shanghai Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Paired-end reads obtained from the original DNA fragments were merged using FLASH (Magoc and Salzberg 2011) and subsequently assigned to each sample using unique barcodes. The sequences were analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) software package (Caporaso et al. 2010), and in-house Perl scripts were employed to analyze beta (between sample) diversity. Initially, the reads underwent filtration using QIIME quality filters. Subsequently, we utilized pick_de_novo_otus.py to select operational taxonomic units (OTUs) and generate an OTU table. Sequences exhibiting ≥ 97% similarity were assigned to the corresponding OTU. A representative sequence was 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, AM fungi, and saprotrophic fungi, using the available data from FUNGuild (Nguyen et al. 2016). The sequencing data for the fungal genomes were deposited in the NCBI Sequence Read Archive (<http://trace.ncbi.nlm.nih.gov/Traces/sra/>) under the accession number PRJNA1002838.

2.5. Data analysis

Soil biota effect (SBE) = $\ln(P_{\text{Live}}/P_{\text{Sterile}})$, where P_{Live} refers to the total biomass of plants in pots without sterilization treatment, and P_{Sterile} refers to the growth traits of plants in sterilized pots. $\text{SBE} > 0$ indicates a positive soil biota effect, while $\text{SBE} < 0$ indicates a negative soil biota effect. Competitiveness index (CI) of plants = $\ln(P_{\text{inter}}/P_{\text{intra}})$, where P_{inter} and P_{intra} refer to the total biomass of plants in pots with interspecific and intraspecific competition, respectively. These formulas were based on the algorithm proposed by Lekberg et al. (2018).

Linear mixed models (LMMs) were used for data analysis, with the response variables being growth traits of plants (total biomass, root biomass, root to shoot ratio, plant height and specific leaf area). The fixed factors were invasive plant presence, microbial treatment (sterilization or not), and biochar addition. Soil source (different invasion areas) were treated as random factors. The effects of soil biota legacy on the growth of invasive and native plants after invasion were analyzed, as well as the restorative effect of biochar on the soil biota legacy. The LMMs analysis was performed using the "lmerTest" package in R software (ver. 3.6.2).

3. Results

3.1. The subsequent plants were improved by the addition of fungicide and biochar

Soil fertilization significantly increased the total biomass and plant height of subsequent plants in the invaded soil (Fig. 2B, S1B). The addition of soil fungicide resulted in a significant reduction in the root-to-shoot ratio of native plants (Fig. 2F) and a concurrent decrease in the specific leaf area of invasive plants (Fig. S1D). The addition of biochar to the invaded soil resulted in a noteworthy enhancement in the root-to-shoot ratio and specific leaf area of indigenous plant species (Fig. 2E, S1C).

3.2. Eukaryotic microbial community in response to soil sterilization and biochar addition

Soil sterilization and the addition of biochar, as well as their interaction, have significant effects on the structure of soil eukaryotic microbial community (Fig. 3A). Soil sterilization led to a 20% increase and a 13% decrease in eukaryotic microbial abundance, respectively. However, the impact of biochar application on the eukaryotic microbial community was only 0.7% (Fig. 3C, D). Biochar application leads to an increase in pathogenic fungi in the soil while suppressing the abundance of mycorrhizal fungi and saprophytic fungi (Fig. S2A, C, E). The soil fungicide reduces the presence of pathogenic fungi in invaded soil and at the same time promotes the recruitment of mycorrhizal fungi and saprophytic fungi by the plants (Fig. S2B, D, F). Conversely, in non-invaded soil, the fungicide promotes the occurrence of soil-borne pathogenic fungi and mycorrhizal fungi, while significantly reducing the abundance of saprophytic fungi.

3.3. Mitigation of biochar on biological legacy effects

The presence of a legacy in the soil following the invasion of exotic plants hampers the biomass accumulation in subsequent invasive plants, resulting in a negative legacy effect (Fig. S3A). The application of biochar to invaded soil mitigates the negative residual effects of soil biota on native plants, while intensifying the negative suppression effect on invasive plants (Fig. S3B). The inclusion of biochar can enhance the competitiveness of native plants while suppressing that of invasive plants (Fig. S4A). The application of biochar enhances the residual effects of eukaryotic microorganisms, and there is a significant negative correlation between the impact of soil residual effects on root systems and the diversity of soil microorganisms (Fig. 4). The utilization of biochar diminishes the impact of residual effects from soil microorganisms on roots (Fig. 5).

4. Discussion

4.1. Soil legacy effects after invasion of alien plants

The remaining soil biota after invasion by alien plants has an inhibitory effect on the biomass accumulation of subsequent plants. Following the introduction of alien plants, their apparent competitive advantage due to the absence of above- and below-ground natural enemies resulted in greater impacts on the soil environment, including soil nutrients, enzyme activity, microbial community structure and diversity, and soil animals (Lau and Lennon 2012). The impact of invasive plants on the soil forms a soil "memory" that continues to influence the growth of subsequent plants. The aim of this study was to analyze the effects of retained soil biota on subsequent plant growth by performing soil sterilization. Soil sterilization reduces the feeding of soil organisms on plant roots and the uptake of root secretions by microorganisms, indirectly reducing the downward secretion of photosynthetic carbon in plants and investing more biomass in the formation of aboveground plant components (Li et al. 2023). In addition, our research has shown that the use of fungicide inhibits the germination of seed banks in the soil, thereby reducing weed competition and indirectly promoting plant growth. The invasive plant *Chromolaena odorata* is known for its high seed production. These huge seeds can germinate rapidly after removal of the invasive plant, hindering the establishment of native plant communities. However, the fungicide used in this study effectively inhibits the germination of the seed bank, creating favourable conditions for the subsequent restoration of the local plant community.

4.2. The eukaryotic microbial community assembly in response to the addition of fungicide and biochar

In our research we have found that fungicides, biochar and their interactions significantly alter eukaryotic microbial communities in soil, with fungicides having the strongest effects (Fig. 3). Soil microbial memory is eliminated by the fungicide and plants then recruit new microbial communities. The soil fungicide impaired the occurrence of pathogenic fungi but promoted the recruitment of mycorrhizal fungi and saprophytic fungi by the plants (Fig. S2). The structure and function of plant rhizosphere microbial community are closely related to the input of plant-derived organic carbon (rhizosphere sedimentary carbon and litter) and soil 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 the use of soil fungicides weakens microbial memory, it directly affects the turnover of nutrients in the soil, which is detrimental to subsequent nutrient uptake by the plants. The addition of biochar replenishes early nutrients, facilitating nutrient uptake and utilization by plants (Hue 2020). Biochar can also absorb some chemicals in the soil and promote plant growth. The combined use of biochar and fungicides exhibits evident interactive effects that have a positive impact on the stability of the subsequent plant, which is due to the stable interaction between plant and soil.

4.3. The remedial effect of biochar on soil legacy effects

The introduction of invasive plants alters the composition of root exudates and litter, selectively stimulating certain soil microorganisms, affecting microbial community structure and function, and influencing the growth of subsequent plants through the "memory" of the soil microbial pool. In addition, invasive plants can also alter the physico-chemical properties of the soil, such as pH, organic matter content and nutrient availability, which in turn can affect microbial communities and the soil biota. Biochar contains some ash elements that can be added directly to plant growth. In addition, the addition of biochar can improve the physical structure of the soil, increase the permeability of the soil, improve the soil environment and indirectly promote plant growth. In addition, biochar has a relatively large specific surface area that can adsorb chemically sensitive substances in the soil, which indirectly promotes plant growth.

Overall, our study highlights the importance of considering the legacy of invasive plants on the soil environment and subsequent plant growth. The soil "memory" created by invasive plants can significantly affect the growth and survival of subsequent plants and have long-term consequences for ecosystem functioning. It is therefore important to take measures to prevent the introduction and spread of invasive plants and to restore and rehabilitate invaded ecosystems to minimize their impact on the soil environment and subsequent plant growth. Converting invasive plants into biochar can completely destroy their seeds and rhizomes, which is beneficial for controlling the spread of invasive plants. The processed biochar provides nutrients, adsorbs chemicals in the soil and improves soil aggregation, which directly promotes plant growth. Additionally, the utilization of biochar effectively alleviates the initial deficiencies of nutrients and root damage caused by fungicides, while simultaneously remediating the environmental pollution resulting from fungicide use. In conclusion, the combined use of biochar and fungicides effectively controls legacy effects caused by invasive plant invasions and has significant practical benefits for invasive plant control and land use.

Conclusions

We used the invasive species *C. odorata* to produce biochar, which was then combined with a fungicide to alleviate the legacy remained in the invaded soil. The soil fungicide reduces the occurrence of pathogenic fungi in invaded soil and at the same time promotes the recruitment of mycorrhizal fungi and

saprophytic fungi by the plants. The incorporation of biochar and fungicide into the soil can significantly reduce the competitive ability of non-native plants while improving the competitive ability of native plants. The application of biochar can effectively mitigate the legacy in the invaded soil and underlines the great importance of soil remediation for the restoration of native plants diversity.

Declarations

Acknowledgements

The authors would like to thank Wen-bian Bo for taking care of the potted plants and Long Li for their assistance in collecting the biomass data. This work was supported by the National Key R&D Program of China (2022YFF1302402) and Yunnan Fundamental Research Projects (202201AT070609), the National Natural Science Foundation of China (32171660, 32071661) and the 14th Five-Year Plan of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (E3ZKFF3B, E3ZKFF9B) and the “Yunnan Revitalization Talent Support Program” in Yunnan Province.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Weitao Li, Hui Ning and Yulong Zheng. The first draft of the manuscript was written by Weitao Li and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

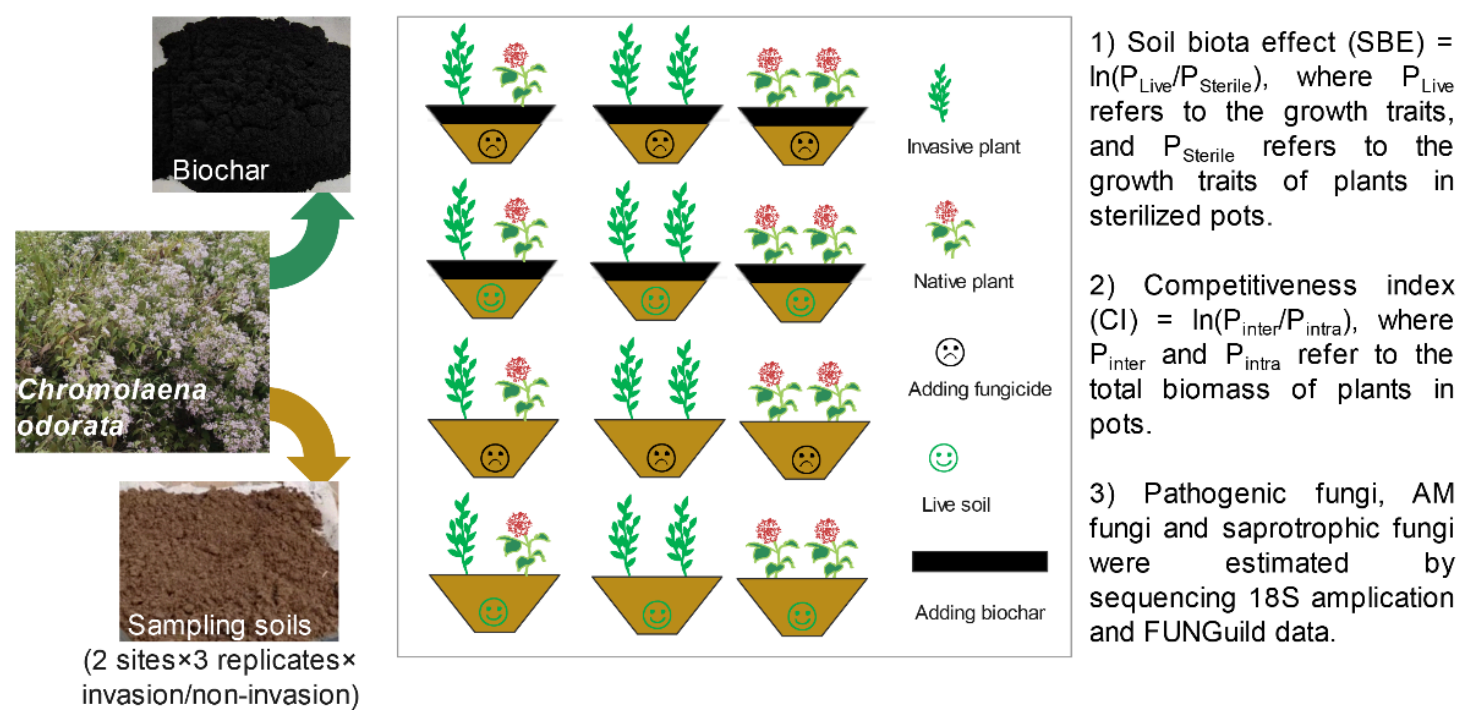


Figure 1

Schematic illustration of experimental layout.

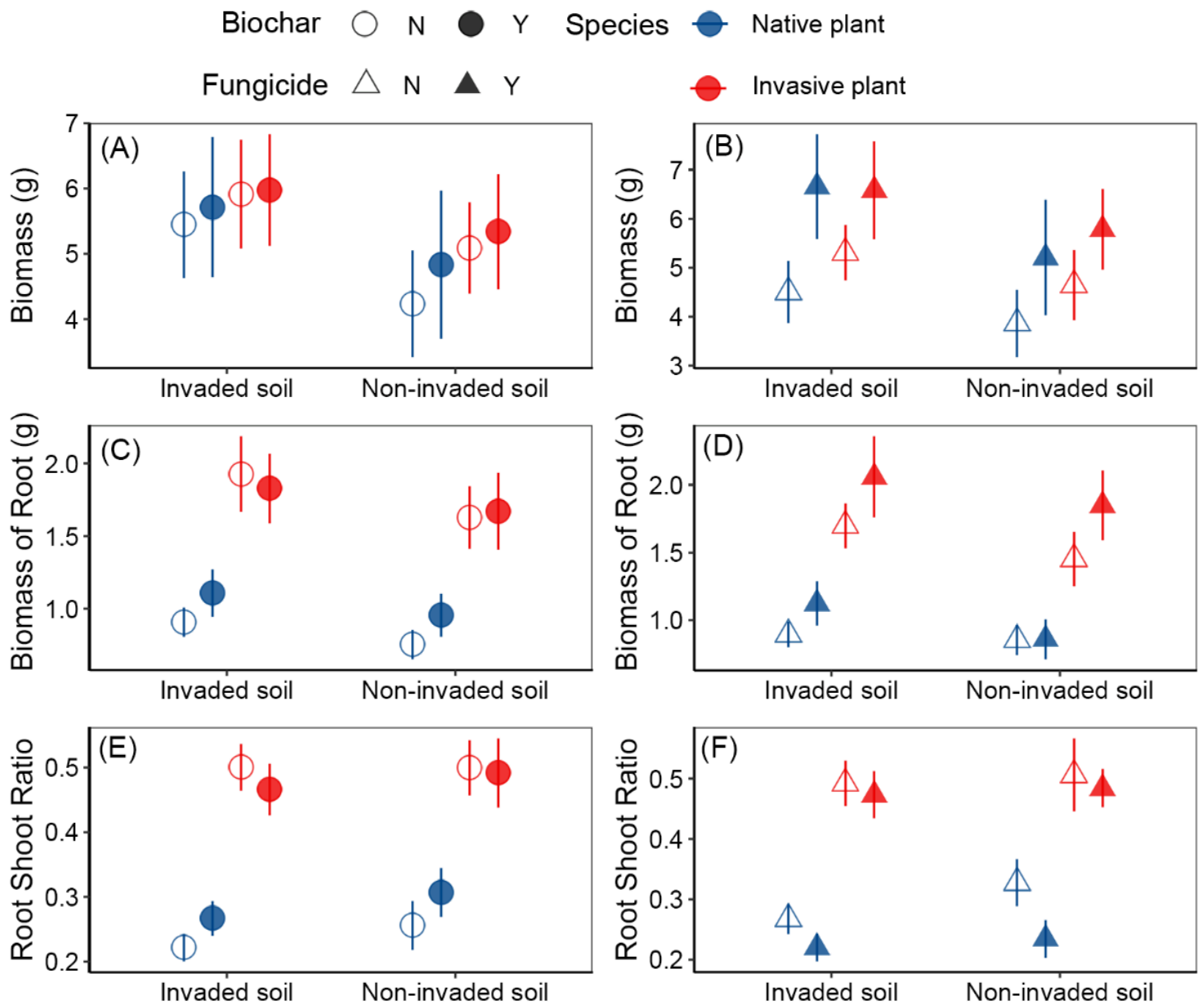


Figure 2

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) and non-addition (N) on subsequent plant biomass. Panels C-D focus on root biomass, while panels E-F examine the root-to-shoot ratio.

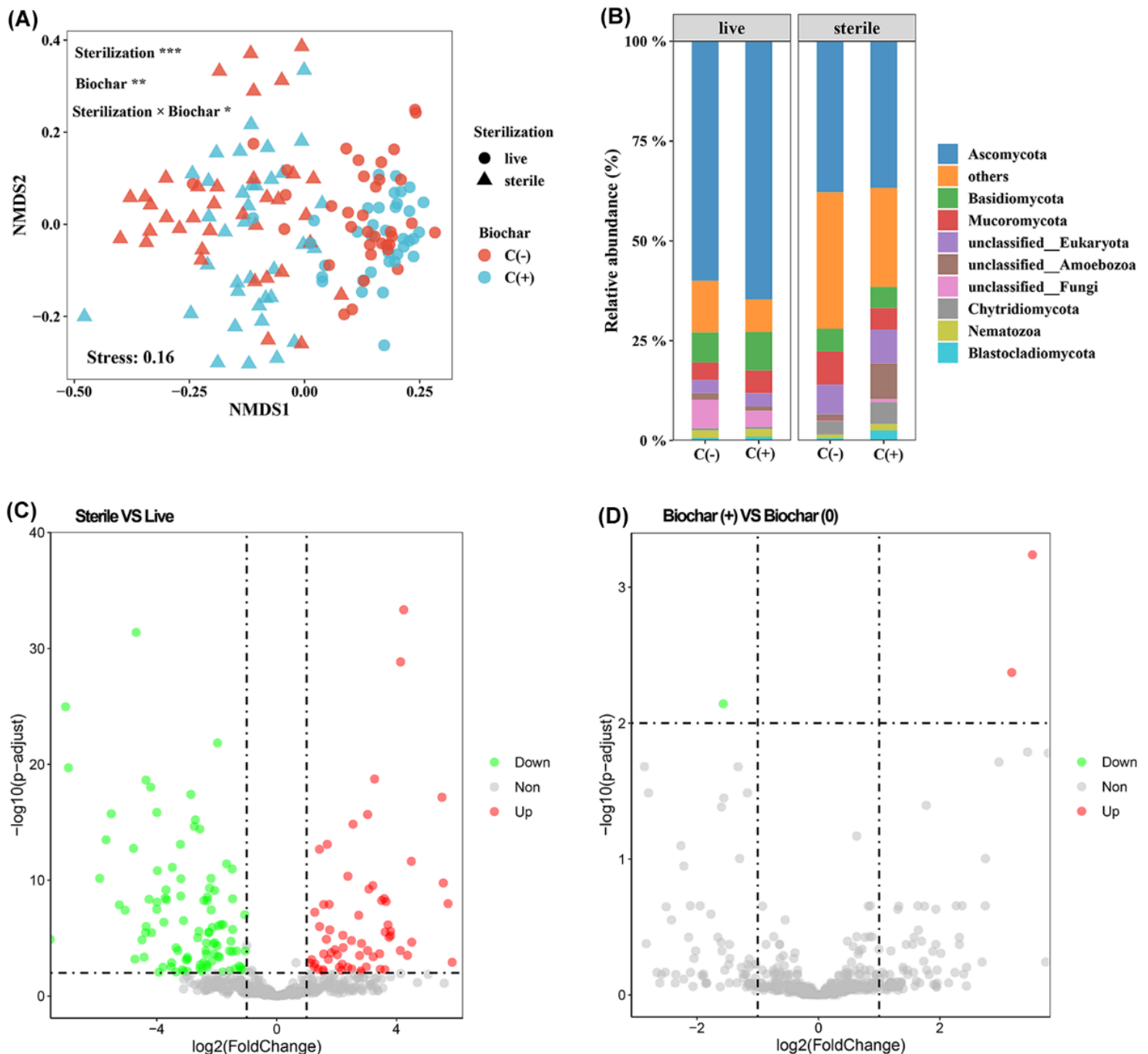


Figure 3

Eukaryotic microbial community in response to the addition of biochar and fungicide. Panels A-B examine the eukaryotic microbial community. Panels C-D investigate the degree of changes induced by the addition of fungicide and biochar in the eukaryotic microbial community. *, **, and *** indicate statistical significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

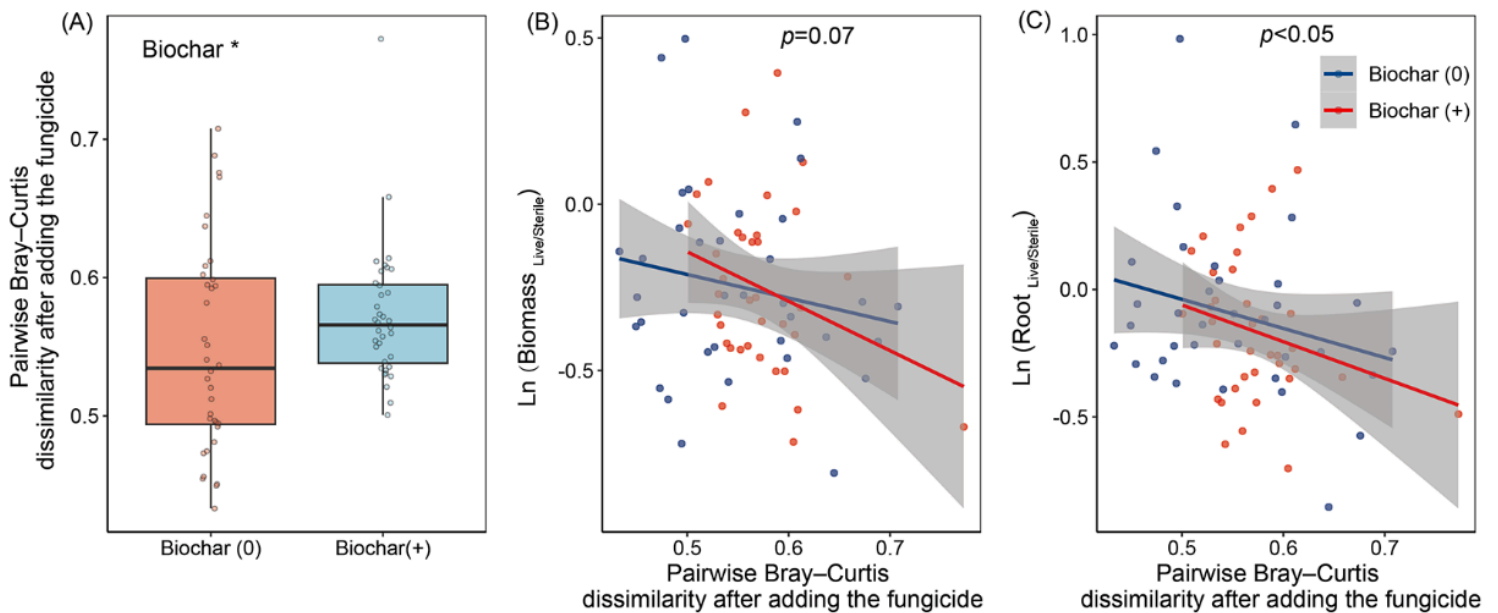


Figure 4

The impact of eukaryotic microbial communities on plant biomass and its distribution following biochar addition. * indicate statistical significance at $p<0.05$.

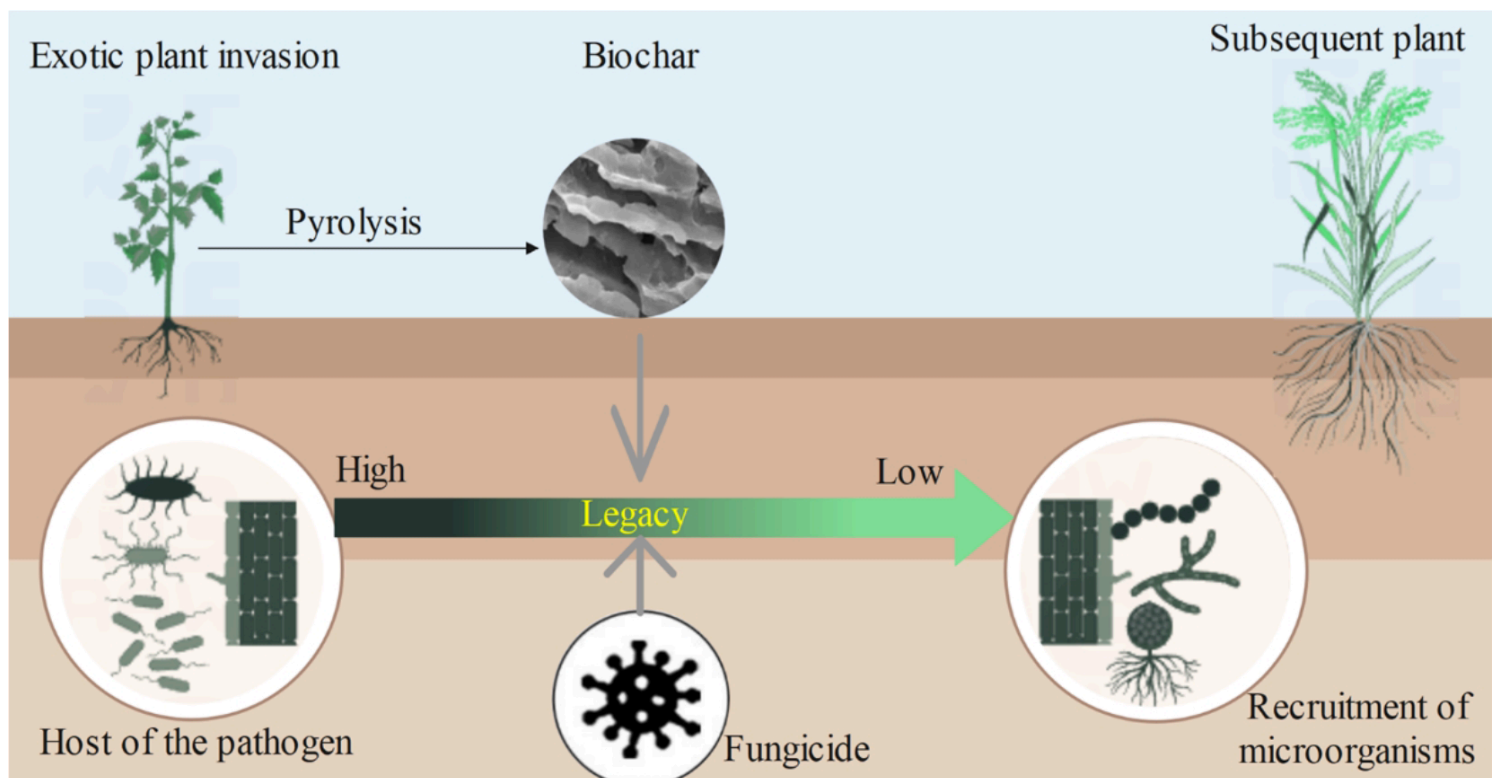


Figure 5

Depicts the concept of remedying eukaryotic microbial legacy effects through the addition of biochar and fungicide.

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