The Impact of Root-Invasive Fungi on Dominant and Invasive Plant Species in Degraded Grassland at Nanshan Pasture

Yanxia Zhang 1, Jiechao Chang 1, Jiayao Xie 1, Liquan Yang 1, Mohamed S. Sheteiwy 2, Abdel-Raouf A. Moustafa 3, Mohamed S. Zaghloul 3 and Haiyan Ren 1,*

1 College of Agro-Grassland Science, Nanjing Agricultural University, Nanjing 210095, China; 2021820026@stu.njau.edu.cn (Y.Z.); 2020120006@stu.njau.edu.cn (J.C.); 2022120010@stu.njau.edu.cn (J.X.); 2022820035@stu.njau.edu.cn (L.Y.)
2 Department of Agronomy, Faculty of Agriculture, Mansoura University, Mansoura 35516, Egypt; salahco_2010@yahoo.com
3 Botany Department, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt; raoufmoustafa2@hotmail.com (A.-R.A.M.); zaghoul_mohamed@yahoo.com (M.S.Z.)
* Correspondence: hren@njau.edu.cn

Abstract: Overgrazing leads to the degradation of grazing lands, which seriously threatens the stability of grassland ecosystems. Root-invading fungi, as one of the main influencing factors, can cause plant diseases in grasslands, reduce the proportion of dominant plant species, increase the proportion of invasive poisonous weeds, and further aggravate degradation. In order to predict and improve the effects of root-invading fungi on grassland degradation, we conducted an in situ soil indoor control experiment using soils collected from non-degraded, moderately degraded, and severely degraded areas of Nanshan pasture in Hunan Province, China. We used monoculture or mixed grasslands of dominant plant species, including Lolium perenne, Trifolium repens, and the invasive weed Persicaria hydropiper, and inoculated them with local strains of pathogenic Fusarium species (Fusarium boothii and Fusarium circinatum) and beneficial fungi Arbuscular Mycorrhizal Fungi (AMF) and Trichoderma hamatum to explore how different strains of fungi affect plant growth and community dynamics. The results showed that Fusarium species (Fusarium boothii and Fusarium circinatum) as a major pathogenic fungus, inhibited the growth of the dominant grass Lolium perenne in moderately and severely degraded soils, which provided growth space and resources for invasive weeds Persicaria hydropiper and further aggravated the degree of grassland degradation. However, the collaborative effect of beneficial fungi (AMF and Trichoderma) and their inhibitory effect on Fusarium species (Fusarium boothii and Fusarium circinatum) could promote the growth of dominant plants and weeds in soils with varying degrees of degradation, which is beneficial to maintaining the stability and diversity of grassland plant communities. The collaborative effect of beneficial fungi could also increase the availability of nutrients in severely degraded soils. Therefore, using beneficial fungi (AMF and Trichoderma) for soil improvement and reducing the harm of pathogenic Fusarium species (Fusarium boothii and Fusarium circinatum) to plant growth is of great significance for promoting the protection and management of grassland ecosystems, as well as for the restoration and recovery of grasslands.

Keywords: degraded grassland; Fusarium; plant community; Trichoderma; arbuscular mycorrhizal fungi

1. Introduction

Grasslands have played a significant role in the development of animal husbandry [1]. However, they are facing severe degradation issues due to overgrazing, leading to a massive invasion of weeds and a dramatic decrease in productivity [2,3]. It is necessary to conduct in-depth research on the mechanisms of weed invasion and take measures to prevent the degradation of grasslands.

Microorganisms are closely associated with weed invasion and may serve as important drivers for successful invasions. Studies have shown that after the invasion of alien weeds, there is a lack of corresponding pathogenic microorganisms in the new habitat, allowing
the successful invasion of the alien weeds [4]. Additionally, when invasive plants enter new habitats, they can affect the growth of native plants. For example, the invasive plant *Chromolaena odorata* can increase the spore population of *Fusarium* in the soil, thus suppressing the growth of two native plants [5]. This phenomenon suggests that invasive plants can become a pathogen reservoir for native plants, severely impacting the ecosystem of native plants. Therefore, studying the interaction between microorganisms and weed invasion is crucial for understanding ecosystem changes during the invasion process. Root-invasive fungi are a group of internal microorganisms that spread through the soil and invade plant roots [6,7]. They have significant impacts on plant growth and development and can parasitize plant roots, resulting in substantial effects [8]. Some of these root-invasive fungi can invade weeds and parasitize their roots, making them alternative hosts for pathogenic fungi and promoting the spread of these pathogens [9,10].

However, current research on the interaction between microorganisms and plants after weed invasion mostly focuses on soil fungi, with relatively little study on root fungi [11–13]. Therefore, further research on the effects of root-invasive fungi on both invasive weeds and native plants, as well as their mechanisms of interaction with invasive weeds and native plants, is of great significance for maintaining grassland ecological balance. In this study, we have selected the dominant local plant *Lolium perenne*, *Trifolium repens*, and the invasive weed *Persicaria hydropiper* as research subjects to investigate the impact of root-invasive fungi on dominant and invasive plants in soils with varying degrees of degradation. This study aims to explore the role of root-invasive fungi in grassland degradation at both the species and community levels, helping us better understand the role and effects of root-invasive fungi in the invasion process and providing a scientific basis for grassland conservation and management.

2. Materials and Methods

2.1. Study Area

The Nanshan Pasture is located 80 km southwest of Chengbu Miao Autonomous County, on the southern edge of western Hunan Province, at 26°12′ N and 109°56′ E. It is a typical high-mountain moss grassland with a total area of 15,300 hectares, an average altitude of 1760 m, and a maximum altitude of 1940 m. The area belongs to the subtropical monsoon humid climate zone of central subtropical mountainous climate, with an average annual temperature of 11 °C, an average annual precipitation of 1218.5 mm, and an average relative humidity of 75% to 83%. The dominant plants in this grassland are *Stipa baicalensis*, *Lolium perenne*, and *Trifolium repens*, while *Potentilla reptans*, *Polygonum hydropiper*, and *Rumex acetosa* L. are subdominant species.

2.2. Preparation of Soil and Tested Fungi

This experiment collected two invasive plant specimens, namely *Rumex acetosa* and *Polygonum hydropiper*, from the degraded grassland of Nanshan pasture for the isolation of root-inhabiting fungi (Unpublished data). Subsequently, the isolated fungi were subjected to pathogenicity testing, and two species of *Fusarium*, namely *Fusarium boothii* and *Fusarium circinatum*, were selected as pathogenic fungi for testing. These two fungi can cause diseases in dominant grasses *Lolium perenne* and *Trifolium repens* in grasslands but have no significant effect on invasive plants such as *R. acetosa* and *P. hydropiper*. In addition, the experiment selected *Trichoderma hamatum*, a beneficial fungus isolated from *R. acetosa*, as a tested beneficial fungus.

According to the national standard “Classification Criteria for Natural Grassland Degradation, Desertification, and Salinization” (GB19377-2003), we assessed the degree of grassland degradation in Nanshan Pasture, Hunan Province. The assessment involved calculating vegetation coverage, aboveground biomass, and the rate of organic matter reduction in the 0–20 cm soil layer. We defined areas where the indicators decreased by 10% as non-degraded (ND) grassland, areas with a decrease of 20–40% as moderately degraded (MD) grassland, and areas with a decrease of over 50% as severely degraded (SD)
grassland. Corresponding soil samples were collected from each degradation zone. During the sampling process, we randomly selected 10 sampling points in each degradation zone with a distance of 10 m between adjacent points. At each sampling point, we used a shovel to collect 10 kg of topsoil (0–10 cm), and in each degradation zone, we collected a total of 100 kg of soil samples. In total, we collected 900 kg of soil samples (100 kg per degradation zone × 3 degradation types × 3 repetitions). The collected soil samples were mixed and sieved (<0.5 cm) in the field to remove visible debris, such as large stones and plant remains, and then transported back to the laboratory for sterilization in a high-pressure sterilizer at 120 °C for 1 h and stored for further analysis.

2.3. Seed and Inoculum Preparation

First, the seeds were disinfected with a 1% NaClO solution for 2 min, then placed on sterilized filter paper in a culture dish and germinated in a 20 °C incubator for later use. Two weeks after seed germination, 5 consistent seedlings from each plant species were randomly selected and transplanted into prepared pots. Then, the pots were placed in the greenhouse of Nanjing Agricultural University according to a completely randomized design.

To prepare the fungal inoculum, the tested fungi (Fusarium boothii, Fusarium circinatum, and Trichoderma hamatum) were cultured on PDA for one week. Three 5 mm diameter fungal discs were taken from the edge of the colony using a sterilized puncher and cultured in LB medium while shaking on a shaker for 3 days. After filtering out the culture medium, the remaining fungal suspension was diluted with sterile water to prepare a fungal suspension with an absorbance of 2, which was used for inoculation.

Propagating and inoculating arbuscular mycorrhizal fungal spores (provided by the School of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, China): Spores of Glomus caledonium are placed in a pot containing sterilized sandy soil with corn as the host for propagation. For inoculation, 20 g of the propagated sandy soil is weighed.

2.4. Greenhouse Experiment

At the species level, the experiment involves 162 pots with 3 different soil degradation levels (non-degraded, moderately degraded, severely degraded), 6 different fungal treatments (Fb, Fc, Fbc, AM, Fbc + AM, and CK), and 3 different plants (Lolium perenne L., Trifolium repens L., and Persicaria hydropiper), each with 3 replicates. Fb represents Fusarium boothii, and Fc represents Fusarium circinatum. Fbc represents a mixture of Fusarium boatii and Fusarium circinatum, AM represents arbuscular mycorrhizal fungi, AM + Fbc represents a mixture of arbuscular mycorrhizal fungi and two Fusarium species, and CK is the control.

At the community level, the experiment involves 72 pots with mixed plants (Lolium perenne L., Trifolium repens L., and Persicaria hydropiper), 3 different soil degradation levels (non-degraded, moderately degraded, severely degraded), and 8 different inoculation treatments (FM, TH, AMF, FM + TH, FM + AMF, AMF + TH, FM + TH + AMF, and CON), each with 3 replicates. FM represents a mixture of Fusarium boothii and Fusarium circinatum, AMF represents arbuscular mycorrhizal fungi, FM + TH represents a mixture of Trichoderma hamatum and two Fusarium species, FM + AMF represents a mixture of arbuscular mycorrhizal fungi and two Fusarium species, AMF + TH represents a mixture of Trichoderma hamatum and arbuscular mycorrhizal fungi, FM + TH + AMF represents a mixture of Trichoderma hamatum, arbuscular mycorrhizal fungi, and two Fusarium species, and CON is the control.

The population experiment selected plastic pots with a diameter of 15 cm and a height of 15 cm, with each pot containing 1500 g of sterilized soil with varying degrees of degradation, for later use. The community experiment selected plastic pots with a diameter of 15 cm and a height of 15 cm, with each pot containing 3000 g of sterilized soil with varying degrees of degradation, for later use. The two experiments were consistent in all other experimental conditions.

Randomly select Pre-germinated seeds were randomly selected, and 5 seedlings with consistent growth were transplanted into each prepared pot. If a seedling died within the
first week of the experiment, it was replaced immediately. The pots were placed in the Nanjing Agricultural University greenhouse according to a completely randomized design, and their positions were changed periodically to ensure even light exposure. The relative humidity was maintained at 70%. Plants received 16 h of light at 21 °C during the day and 8 h of light at 16 °C during the night, and were watered every other day. The initial soil moisture was measured twice a week (calculated as 17% of soil dry weight).

2.5. Harvesting and Measurements

After 18 weeks, the aboveground plants of all pots in both the species-level and community-level experiments were collected, dried in an oven (70 °C for 3 days), and weighed. The soil samples from the community-level experiment were air-dried in a ventilated area after passing through a 2 mm sieve for the determination of basic soil physical and chemical properties. The soil samples were extracted using 2 mol/L KCl, and nitrate nitrogen (NO$_3^-$-N) was determined using colorimetry (at 220 nm and 275 nm) [14]. Ammonium nitrogen (NH$_4^+$-N) was determined using colorimetry (at 625 nm) after extraction with indophenol blue-potassium chloride [14]. The available phosphorus content of the soil samples was measured using the Olsen method (mg/g) [15]. Finally, the organic carbon content (SOC) of the soil samples was directly determined using a TOC analyzer [16].

2.6. Data Analysis and Statistical Analysis

All data were processed using Excel, and statistical analyses were performed after calculating the mean values of different observations within the same replication. A three-way ANOVA was conducted to evaluate the effects of plant species, degradation level, fungal treatment, and their interactions on aboveground biomass. A two-way ANOVA was used to examine the effects of degradation level, fungal treatment, and their interactions on total biomass and soil nutrients (organic carbon, available phosphorus, ammonium nitrogen, and nitrate nitrogen). The statistical analyses were conducted using IBM SPSS 22. In this paper, all significance levels were indicated at the 0.05 level. Graphs were generated using Origin2021.

3. Results

3.1. Effects of Pathogenic Fungi on Plant Biomass

Degradation level and fungal treatment had significant effects on the aboveground biomass of different plants (Table 1). The aboveground biomass of all three plants showed a decreasing trend with increasing degradation level (Figure 1). There was an interaction between degradation level and fungal treatment on the aboveground biomass of Lolium perenne, Persicaria hydropiper, and Trifolium repens L. (Table 1). Compared with the CK control, Fb, Fc, Fbc, and Fbc + AM decreased the aboveground biomass of Lolium perenne and Trifolium repens L. but had no significant effect on the aboveground biomass of Persicaria hydropiper in non-degraded and moderately degraded soils (Figure 1); among them, the aboveground biomass of Lolium perenne was lowest under the Fbc fungal treatment, and the aboveground biomass of Trifolium repens L. was lowest under the Fbc, Fb, and Fc fungal treatments. In severely degraded soils, Fb, Fc, Fbc, and AM + Fbc reduced the aboveground biomass of all three plants.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DF</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant species (P)</td>
<td>2</td>
<td>208.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Degree of degradation (D)</td>
<td>2</td>
<td>50,746.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fungal treatment (F)</td>
<td>5</td>
<td>6696.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P × D</td>
<td>4</td>
<td>32.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P × F</td>
<td>10</td>
<td>501.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D × F</td>
<td>10</td>
<td>4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P × D × F</td>
<td>20</td>
<td>3.6</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Arbuscular mycorrhizal fungi. Different uppercase letters indicate significant differences in different degrees of degradation (FM + AMF and FM + TH + AMF) (Figure 2).

The effects of different levels of soil degradation and fungal treatments on aboveground biomass of all three plants. In severely degraded soils, Fb, Fc, Fbc, and AM + Fbc reduced the aboveground biomass of the community treatments. In severely degraded soils, the community aboveground biomass treated with pathogenic fungi (FM) is significantly lower than other treatments (TH, AMF, FM + TH, FM + AMF, and CON). In all three degraded soils, fungal treatment (TH, AMF, FM + TH + AMF, and CON). In all three degraded soils, fungal treatment (TH, AMF, FM + TH + AMF, and CON). In all three degraded soils, fungal treatment (TH, AMF, FM + TH, FM + AMF, and FM + TH + AMF) increases the community biomass, and the highest increase is observed under mixed treatment with pathogenic and beneficial fungi (FM + AMF and FM + TH + AMF) (Figure 2).

### 3.2. Community Total Biomass

Both degradation level and fungal treatment have significant effects on the aboveground biomass of the community (Table 2). The aboveground biomass of the community decreases with increasing degradation level (Figure 2). In moderately degraded and severely degraded soils, the community aboveground biomass treated with pathogenic fungi (FM) is significantly lower than other treatments (TH, AMF, FM + TH, FM + AMF, FM + TH + AMF, and CON). In all three degraded soils, fungal treatment (TH, AMF, FM + TH, FM + AMF, and FM + TH + AMF) increases the community biomass, and the highest increase is observed under mixed treatment with pathogenic and beneficial fungi (FM + AMF and FM + TH + AMF) (Figure 2).

Table 2. The effects of different levels of soil degradation and fungal treatments on aboveground biomass (AB), soil organic carbon (SOC), soil inorganic nitrogen (NH$_4^+$ and NO$_3^-$), and available phosphorus (AP) in the soil community were studied using a two-way analysis of variance (ANOVA). The results provide F-values (F) and p-values (p), where (p < 0.05) indicates a significant difference and (p < 0.01) indicates a highly significant difference.

<table>
<thead>
<tr>
<th>Treatment Type</th>
<th>DF</th>
<th>AB</th>
<th>F</th>
<th>p</th>
<th>SOC</th>
<th>F</th>
<th>p</th>
<th>NO$_3^-$</th>
<th>F</th>
<th>p</th>
<th>NH$_4^+$</th>
<th>F</th>
<th>p</th>
<th>AP</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree of degradation</td>
<td>2</td>
<td>170,582.298</td>
<td>&lt;0.001</td>
<td>3492.557</td>
<td>&lt;0.001</td>
<td>73.577</td>
<td>&lt;0.01</td>
<td>11.169</td>
<td>&lt;0.001</td>
<td>770.432</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungal treatment (F)</td>
<td>7</td>
<td>221,834</td>
<td>&lt;0.001</td>
<td>16,955</td>
<td>&lt;0.001</td>
<td>4.595</td>
<td>&lt;0.001</td>
<td>3.252</td>
<td>&lt;0.01</td>
<td>7.571</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D × F</td>
<td>14</td>
<td>8,840</td>
<td>&lt;0.001</td>
<td>6,121</td>
<td>&lt;0.001</td>
<td>0.469</td>
<td>0.938</td>
<td>0.056</td>
<td>0.885</td>
<td>0.735</td>
<td>0.730</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Changes in aboveground biomass of three plant species ((a) Lolium perenne, (b) Trifolium repens L., and (c) Persicaria hydropiper) after inoculation with fungi. Fb: Fusarium boothii, Fc: Fusarium circinatum, AM: Arbuscular mycorrhizal fungi. Different uppercase letters indicate significant differences in different degrees of degradation (p < 0.05), and different lowercase letters indicate significant differences in different fungal inoculation treatments under the same degree of degradation (p < 0.05).
Figure 2. Changes in aboveground biomass of plant community after inoculation with fungi in different degrees of degradation. Different uppercase letters indicate significant differences in different degrees of degradation ($p < 0.05$), and different lowercase letters indicate significant differences in different fungal inoculation treatments under the same degree of degradation ($p < 0.05$).

### 3.3. The Biomass of Each Plant Species in the Community

The biomass of three plant species, namely *Lolium perenne*, *Trifolium repens*, and *Polygonum hydropiper*, decreased with increasing degradation degree under different fungal treatments, consistent with the results at the species level (Figure 3). In the same degraded soil, different fungal inoculations had different effects on the biomass of different plants. In contrast to the results in the previous chapter, the pathogenic fungal treatment (FM) significantly reduced the aboveground biomass of *L. perenne* in moderately and severely degraded soils but had no significant effect on *T. repens* and *P. hydropiper*. In different degraded soils, inoculation with different fungi (TH, AMF, FM + TH, FM + AMF, TH + AMF, and FM + TH + AMF) increased the biomass of the three plant species to varying degrees (Figure 3), but the TH + AMF treatment resulted in the most significant increase in biomass for the three plant species across different degraded soils. Furthermore, the aboveground biomass of *L. perenne*, *T. repens*, and *P. hydropiper* was significantly higher under the FM + TH + AMF treatment than under the FM treatment, indicating that the promotion of AMF and *Trichoderma* on the three plant species was greater than the inhibitory effect of *Fusarium*.

Figure 3. Changes in aboveground biomass of plant species in different degraded soils after inoculation with fungi. *Lolium Perenne* (a), *Trifolium repens* (b), and *Polygonum hydropiper* (c); FM: *Fusarium borthi* + *Fusarium circinatum*, Th: *Trichoderma hamatum*, AMF: arbuscular mycorrhizal fungi. Different uppercase letters indicate significant differences in different degrees of degradation ($p < 0.05$), and different lowercase letters indicate significant differences in different fungal inoculation treatments under the same degree of degradation ($p < 0.05$).
3.4. Soil Nutrients

Degradation and fungal treatments both significantly affect soil organic carbon, ammonium nitrogen, and available phosphorus content but only have an interactive effect on soil organic carbon content (Table 2). Under different fungal treatments, soil organic carbon content decreased with increasing degradation degree (Figure 4). In soils with different degrees of degradation, the beneficial fungus (AMF + TH) significantly increased soil organic carbon, ammonium nitrogen, and available phosphorus content compared to the untreated control (CON), while the pathogenic fungus (FM) only significantly decreased soil organic carbon content in moderately degraded soil, without significant effect on soil nitrate nitrogen and available phosphorus content (Figure 4).

![Figure 4](image_url)

**Figure 4.** Changes in soil nutrients of plant communities under different degradation degrees after inoculation with fungi. (a) Organic carbon, (b) available phosphorus, (c) ammonium nitrogen, and (d) nitrate nitrogen; FM: *Fusarium boothii* + *Fusarium circinatum*, TH: *Trichoderma hamatum*, AMF: arbuscular mycorrhizal fungi. ns indicates no significant difference among degraded soils. Different uppercase letters indicate significant differences in different degrees of degradation (p < 0.05), and different lowercase letters indicate significant differences in different fungal inoculation treatments under the same degree of degradation (p < 0.05).

4. Discussion

In our study, we found that the parasitic effects of the invading weed fungi *Persicaria hydropiper* and *Rumex acetosa* derived *Fusarium* on plants vary at different ecological levels, including the species level and the community level. At the species level, *Fusarium* can inhibit the growth of multiple plant species, while at the community level, it significantly affects the growth of *Lolium perenne*. Regardless of the level, *Fusarium* significantly inhibits the growth of the dominant plant species, *Lolium perenne*. Further research indicates that the combined action of symbiotic fungi such as AMF or *Trichoderma* can suppress the harmful effects of *Fusarium* on both dominant plant species and invading weeds while improving the availability of nutrients in degraded grassland soils. Therefore, we believe that root fungi from invading weeds exacerbate grassland degradation by suppressing the
growth of native plants, and introducing symbiotic fungi can be an effective biocontrol measure to aid in the restoration of degraded grassland ecosystems.

4.1. Effects of Pathogenic Fungi on Plants

Pathogenic strains of *Fusarium* can parasitize multiple plant hosts, leading to a reduction in plant diversity [5,17]. In this study, we found that in non-degraded and moderately degraded soils, pathogenic strains of *Fusarium* isolated from the invading weeds *Persicaria hydropiper* and *Rumex acetosa*—when inoculated alone or in combination—reduced aboveground biomass of the dominant native plants *Lolium perenne* and *Trifolium repens*. The inhibitory effect was more significant with mixed inoculation but had no significant impact on the invading weeds. However, in severely degraded soils, *Fusarium* exhibited varying degrees of inhibition on both dominant native plants (such as *Lolium perenne* and *Trifolium repens*) and invading plants (*Polygonum hydropiper*). This indicates that the pathogenic strains of *Fusarium* isolated from the invading weeds *Persicaria hydropiper* and *Rumex acetosa* infect host plants in response to soil conditions, and the combined infection of *Fusarium* has a more pronounced inhibitory effect on plant growth [18].

According to the disease triangle theory, the parasitic relationship between pathogens and plants is closely related to soil conditions [19]. The results of this study further demonstrate that in severely degraded soils, pathogenic *Fusarium* infected a greater number of plant species, indicating a stronger inhibitory effect on plants in heavily degraded soils. Furthermore, the study results also indicate that in severely degraded soils, *Fusarium* has a significant impact on the aboveground biomass of *Lolium perenne* and *Trifolium repens* but has no significant effect on the invading plants. This suggests that the effect of *Fusarium* on plants varies depending on the plant species. Previous research has shown that invading weeds can thrive in nutrient-poor soils and possess strong adaptability [20,21]. Consistent with previous studies, our findings indicate that in degraded grasslands, invading weeds exhibit a stronger resistance to *Fusarium*. In contrast, invading weeds are better adapted to degraded soil conditions and can withstand pathogen infection more effectively.

4.2. The Role of Pathogenic Fungi in Plant Communities

A high nutrient supply can counterbalance the negative impact of soil pathogens on plants and maintain the stability of plant communities [22]. In this study, inoculation with two strains of *Fusarium boothii* and *Fusarium circinatum* resulted in a decrease in biomass in plant communities of different degraded soils. Compared to the control group without inoculation, the biomass was reduced by 1.14%, 2.8%, and 9.6% in non-degraded, moderately degraded, and severely degraded soil, respectively. These findings suggest that the root fungi *Fusarium boothii* and *Fusarium circinatum*, found in the roots of invasive weeds, may exhibit a more pronounced inhibitory effect on plant communities in severely degraded soils. Consistent with previous research, the lower nutrient content in degraded soils may enhance the negative impact of the root fungi *Fusarium boothii* and *Fusarium circinatum* on plants.

In degraded grasslands, the competitive ability of native dominant grasses is reduced, leading to a significant decrease in their proportion. The proportion of invasive weeds increases significantly, replacing native dominant grasses as the dominant plants in degraded grasslands [23,24]. In this study, co-inoculation with *Fusarium boothii* and *Fusarium circinatum* in different degraded soils resulted in a decrease in the total biomass of the plant community. In moderately and severely degraded soils, the biomass of the dominant grass *Lolium perenne* was significantly reduced, while the biomass of *Trifolium repens* and *Polygonum hydropiper* was unaffected. This indicates that the decrease in total biomass of the community is due to the inhibitory effect of *Fusarium boothii* and *Fusarium circinatum* on *Lolium perenne* growth. The inhibition of *Lolium perenne* by *Fusarium boothii* and *Fusarium circinatum* reduces its competitive ability in the grassland, further diminishing the proportion of native dominant grasses and providing growing space for weed invasion.

In plant communities, the interactions between different plants alter the relationship between pathogenic fungi and hosts [25]. Our study also found differences in the parasitic
effects of the root fungi *Fusarium boothi* and *Fusarium circinatum* on plants at the species and community levels. At the species level, *Fusarium boothi* and *Fusarium circinatum* can inhibit the growth of multiple plant species, while at the community level, they only significantly inhibit the growth of *Lolium perenne*. This indicates that the interactions between *Lolium perenne*, *Trifolium repens*, and *Polygonum hydropiper* affect the relationship between *Fusarium boothi* and *Fusarium circinatum* and their hosts. However, whether at the species level or the community level, *Fusarium boothi* and *Fusarium circinatum* have a significant inhibitory effect on the growth of the dominant plant *Lolium perenne*. Therefore, we conclude that the inhibition of *Lolium perenne* by *Fusarium boothi* and *Fusarium circinatum* is one of the important factors contributing to the degradation of the Nanshan pasture [26]. Specifically, in the undegraded grassland of the Nanshan pasture, *Lolium perenne* is the dominant plant and is usually able to occupy more resources and space, while the weed *Polygonum hydropiper* is a subdominant plant that needs to grow in intense competition. *Fusarium boothi* and *Fusarium circinatum* reduce the competitive advantage of *Lolium perenne*, providing growing space and resources for invasive weeds such as *Polygonum hydropiper*, which favors the survival and reproduction of invasive weeds and ultimately leads to grassland degradation.

### 4.3. The Role of Symbiotic Fungi in the Community

Symbiotic fungi are closely related to plant growth and can influence plant nutrient absorption, compete for soil nutrients, decompose soil organic matter, and ultimately determine plant yield [27–29]. Our research shows that under different levels of degradation, inoculating AMF and *Trichoderma* increased the total biomass of the community by 5.7%, 4.8%, and 18% in non-degraded, moderately degraded, and severely degraded soil, respectively. This indicates that AM fungi and *Trichoderma* had a positive effect on plant communities in different degraded soils, and the promoting effect on plant communities was strongest in severely degraded soils [30]. This is consistent with previous studies that showed symbiotic fungi (AMF and *Trichoderma*) can promote plant growth, and this growth-promoting effect is stronger in nutrient-poor soils, indicating that the combined action of symbiotic fungi (AMF and *Trichoderma*) can promote the restoration of degraded grasslands and maintain community stability [31,32]. At the same time, symbiotic fungi can suppress the harmful effects of pathogenic microorganisms on plants. In this study, compared to inoculating only with *Fusarium boothi* and *Fusarium circinatum*, inoculating with MX (two *Fusarium* species + AMF + *Trichoderma*) significantly increased the community biomass, which may be because symbiotic fungi (AMF and *Trichoderma*) offset the negative effects of pathogenic fungi on plants. In addition, previous studies have shown that AMF and *Trichoderma* can increase the availability of soil nutrients by decomposing soluble or insoluble organic matter. In this experiment, AMF or *Trichoderma* increased the content of soil organic carbon, available phosphorus, and nitrate nitrogen, and the mixed treatment of symbiotic fungi (AMF and *Trichoderma*) significantly increased the availability of soil nutrients, indicating that the combined action of AMF and *Trichoderma* can produce a synergistic effect that improves soil nutrient limitations.

### 5. Conclusions

In conclusion, the results showed that the inhibition of the dominant plant *Lolium perenne* by two *Fusarium* species (*Fusarium boothi* and *Fusarium circinatum*) was one of the important causes of grassland degradation in Nanshan, and this inhibition is not conducive to the regeneration of degraded grassland *Lolium perenne*. However, the mixed application of AMF and *Trichoderma hamatum* can promote the growth of dominant plants and weed plants, reduce the harm of *fusarium* to plants, and increase the availability of soil nutrients; thus, grassland restoration and reconstruction can be achieved.

**Author Contributions:** H.R. designed the experiment, H.R. and Y.Z. performed the experiment, and all the authors contributed to writing the manuscript. All authors have read and agreed to the published version of the manuscript.
Funding: This project was supported by the National Natural Science Foundation of China (32271615), the Fundamental Research Funds for the Central Universities (KYCYXT2022008), and XUEKEN20220220.

Data Availability Statement: Please contact the first author with requests for data.

Acknowledgments: We are grateful to all the students who assisted with the collection and processing of data. Many thanks are expressed to the anonymous reviewers for their helpful suggestions.

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

References